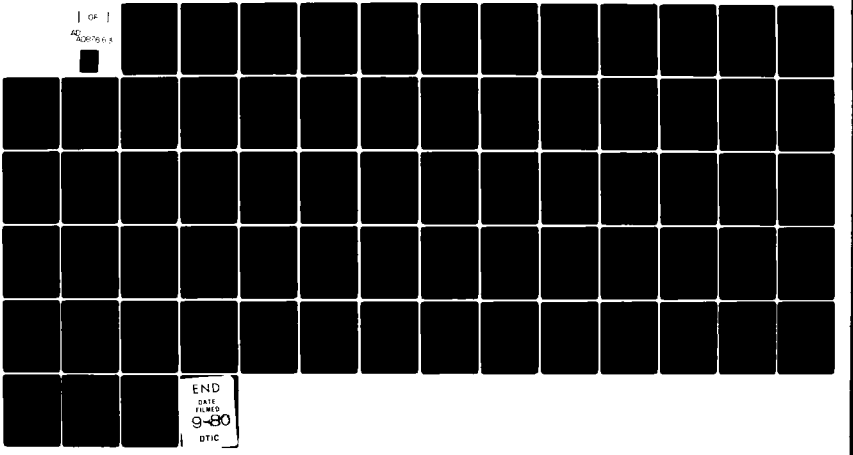
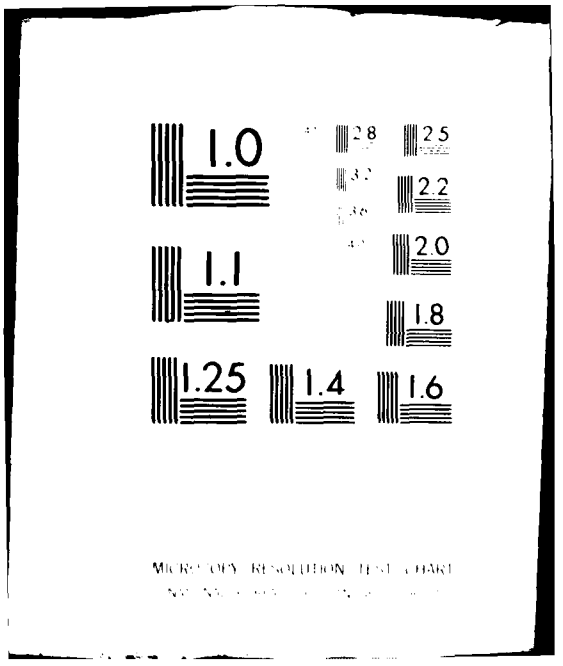
AD-A087 683 WATER AND AIR RESEARCH INC GAINESVILLE FL F/G 13/2
A SUMMARY AND EVALUATION OF AQUATIC ENVIRONMENTAL DATA IN RELAT--ETC(U)
MAR 79 J H SULLIVAN, H D PUTNAM, M A KEIRN DAMD17-77-C-7027
UNCLASSIFIED NL




1.0
1.1
1.25
1.4
1.6
1.8
2.0
2.2
2.5
2.8
MICROCOPY RESOLUTION TEST CHART

LEVEL II (12)

AD_____

A SUMMARY AND EVALUATION OF AQUATIC ENVIRONMENTAL DATA IN RELATION TO ESTABLISHING WATER QUALITY CRITERIA FOR MUNITIONS-UNIQUE COMPOUNDS

PART 4: RDX AND HMX

FINAL REPORT

J.H. SULLIVAN, JR., H.D. PUTNAM, M.A. KEIRN, B.C. PRUITT, JR., J.C. NICHOLS, AND J.T. McCLAVE

(11) MARCH 1979

Supported by

U.S. ARMY MEDICAL RESEARCH & DEVELOPMENT COMMAND

Ft. Detrick, Frederick, MD 21701

J. GARETH PEARSON, PROJECT OFFICER
CONTRACT NO. DAMD 17-77-C-7027

WATER AND AIR RESEARCH, INC.
Gainesville, FL 32602

APPROVED FOR PUBLIC RELEASE: DISTRIBUTION UNLIMITED

THE FINDINGS IN THIS REPORT ARE NOT TO BE CONSTRUED AS AN OFFICIAL DEPARTMENT OF THE ARMY POSITION UNLESS SO DESIGNATED BY OTHER AUTHORIZED DOCUMENTS.

SECURITY CLASSIFICATION OF THIS PAGE (When Data Entered)

REPORT DOCUMENTATION PAGE	READ INSTRUCTIONS BEFORE COMPLETING FORM
1. REPORT NUMBER	2. GOVT ACCESSION NO. AD-087683 / 3. RECIPIENT'S CATALOG NUMBER
4. TITLE (and Subtitle) A SUMMARY AND EVALUATION OF AQUATIC ENVIRONMENTAL DATA IN RELATION TO ESTABLISHING WATER QUALITY CRITERIA FOR MUNITIONS UNIQUE COMPOUNDS	5. TYPE OF REPORT & PERIOD COVERED Final - RDX and HMX 6. PERFORMING ORG. REPORT NUMBER
7. AUTHOR(s) J. H. Sullivan, Jr., H. D. Putnam, M. A. Keirn, J. C. Nichols, and J. T. McClave	8. CONTRACT OR GRANT NUMBER(s) DAMD17-77-C-7207
9. PERFORMING ORGANIZATION NAME AND ADDRESS Water and Air Research, Inc. P.O. Box 1121 Gainesville, FL 32602	10. PROGRAM ELEMENT, PROJECT, TASK AREA & WORK UNIT NUMBERS 62720A.3E162720A835.00.067
11. CONTROLLING OFFICE NAME AND ADDRESS US Army Medical Research and Development Command Ft. Detrick Frederick, MD 21701	12. REPORT DATE March 1979 13. NUMBER OF PAGES 71
14. MONITORING AGENCY NAME & ADDRESS(if different from Controlling Office)	15. SECURITY CLASS. (of this report) Unclassified 15a. DECLASSIFICATION/DOWNGRADING SCHEDULE

16. DISTRIBUTION STATEMENT (of this Report)

Approved for public release; distribution unlimited

17. DISTRIBUTION STATEMENT (of the abstract entered in Block 20, if different from Report)

18. SUPPLEMENTARY NOTES

19. KEY WORDS (Continue on reverse side if necessary and identify by block number)

HMX, RDX, cyclotrimethylenetrinitramine, cyclotetramethylenetetranitramine CAS No. 121-82-4 CAS No. 2691-41-0, water quality criteria

20. ABSTRACT (Continue on reverse side if necessary and identify by block number)

~~The purpose of this report is to review~~ the effects of RDX and HMX on the aquatic environment and ~~to recommend~~ water quality criteria for the protection of aquatic organisms ~~are recommended~~.

Chemical properties, analytical methods, manufacturing wastewater characteristics, and environmental fate of RDX and HMX are reviewed and discussed.

DD FORM 1 JAN 73 1473 EDITION OF 1 NOV 65 IS OBSOLETE

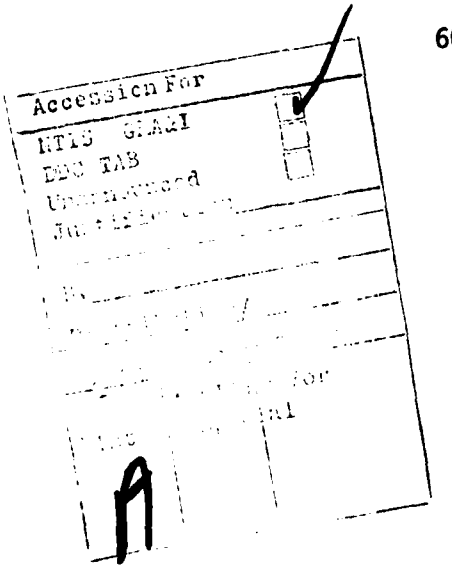
SECURITY CLASSIFICATION OF THIS PAGE (When Data Entered)

The data base for RDX consists of acute tests with four species of freshwater algae, acute and chronic tests with four and two species of freshwater invertebrates respectively, and acute and chronic tests with four and two species of freshwater fish respectively.

The data base for HMX is more limited consisting of acute tests on four species of freshwater algae, acute tests on four species of freshwater invertebrates, and acute tests on four species of freshwater fish. Effects were observed only at nominal concentrations exceeding the solubility of HMX.

Three procedures were utilized to determine the recommended water quality criteria for RDX and HMX; (1) a proposed procedure by EPA, (2) acute toxicity values multiplied by a general application factor, and (3) acute toxicity values multiplied by an experimentally derived application factor. For RDX, a 24-hour average concentration of 0.30 mg/L should adequately protect aquatic life. Insufficient data exist to establish a criteria for HMX.

TABLE OF CONTENTS



LIST OF TABLES

LIST OF TABLES (continued)

LIST OF FIGURES

I. Introduction

Since the early part of the 1970's the U.S. Army Medical Research and Development Command has been supporting research to determine the environmental hazards associated with wastewater discharges from its munitions industry. The objective of these studies has been to develop the scientific data base from which the environmental hazards of munitions unique materials could be assessed.

The purpose of this report is to review the environmental effects of two nitramine explosives, cyclotrimethylenetrinitramine (RDX) and cyclotetramethylenetetranitramine (HMX), on the aquatic environment and to recommend water quality criteria for the protection of aquatic organisms. The two compounds are reviewed together because of their chemical similarity and the probability that they will both be governed by the same criteria. Both compounds are prepared by nitration of hexamethylenetetramine in the presence of ammonium nitrate and acetic anhydride. The explosive produced can be enriched for either HMX or RDX by adjusting the reaction conditions. The widest application of RDX and HMX in military munitions has been as burster charges for artillery shells. HMX has also been used as a component of solid-fuel rocket propellants and to implode fissionable material in nuclear devices to achieve critical mass. Mixtures of RDX and/or HMX with special plasticizers and solvents give rise to plastic explosives designated as "composition C", PBX, and PBXN.

The two compounds are high explosives with approximately 130 percent the explosive power of trinitrotoluene (TNT) (Mark et al. 1965). Since both RDX and HMX are shock-sensitive they are generally cast as dispersions either in wax ("composition A" explosives) or in TNT to form mixtures termed "cyclotol", "octols" or "composition B" explosives (Small and Rosenblatt 1974, Glennon et al. 1977).

HMX, RDX, and the various explosive formulations derived from them are produced at Holston Army Ammunition (HAAP), Kingsport, Tennessee (Small and Rosenblatt 1974). These compounds are released into the environment during the manufacturing and blending processes at this plant, and from load, assemble, and pack (LAP) plants where these materials are loaded into bombs and projectiles. LAP wastewaters also contain TNT and other materials and are currently under separate investigation. The RDX/HMX manufacturing process also generates certain nitramine byproducts, waste cyclohexanone, and cyclohexanone related impurities. The occurrence of these additional components in wastewaters is under separate investigation. The RDX/HMX manufacturing process also generates certain nitramine byproducts, waste cyclohexanone, and cyclohexanone related inpurities. The occurrence of these additional components in wastewaters is under separate investigation (Glennon et al. 1977 and Kitchens et al. 1978). This report will therefore focus primarily on the effects of RDX and HMX. The impact of these compounds has been examined by assessing vertebrate, invertebrate, and algal toxic response by conducting laboratory bioassays. In addition, field investigations have examined the response of selected aquatic communities to these substances.

Recommended acute and chronic safe levels for these compounds in water were developed using the methods recommended by the American Public Health Association (1975), the National Academy of Sciences (NAS) (1973), and the Environmental Protection Agency (EPA) (1976 and 1978) methodology. The latter three documents contain the strategy necessary to provide application factors for predicting environmentally safe levels based on laboratory bioassays.

II. Chemical and Physical Properties

RDX and HMX are explosive polynitramine compounds that have been extensively used in military munitions formulations during and since World War II. Both compounds are prepared by nitration of hexamethylenetetramine (hexamine) by HNO_3 and NH_4NO_3 in the presence of acetic anhydride and acetic acid. RDX and HMX are cyclic secondary nitramines and therefore have somewhat similar chemical and physical properties. Both are colorless or white crystalline solids with specific gravities of 1.82 and 1.87 (RDX and HMX, respectively). They both contain the same proportions of carbon, nitrogen, hydrogen, and oxygen. They are nearly insoluble in water but soluble in organic reagents. In general RDX is more soluble than HMX. Table 1 and Table 2 summarize the general properties of each of the two compounds. Figure 1 shows their structural formulae along with those of hexamine, hexahydro-1, 3-dinitro-5 acetyl-s-triamine (TAX), and octahydro-1-acetyl-3,5,7-trinitro-s-tetramine (SEX). TAX and SEX are byproducts resulting from RDX/HMX manufacturing and are present in RDX/HMX wastewaters. Both of these acetylated nitramines are more water-soluble than RDX and HMX (Kitchens et al. 1978). Levels of TAX and SEX in HAAP wastewaters appear to be 60-90 and 20-40 percent, respectively, of the RDX concentrations (Kitchens et al. 1978). The toxicity and environmental fate of these compounds have not been extensively studied, however, and many of their chemical and physical properties are only inferred from those of RDX and HMX. Kitchens et al. (1978) have summarized the available information pertinent to TAX and SEX.

Both RDX and HMX share a common manufacturing process and wastewater discharges. HMX always appears as a contaminant of RDX and is present in environmental discharges resulting from RDX production and warhead loading. In fact, HMX is present in higher concentrations in wastewater discharged in RDX manufacture than in HMX manufacture (Kitchens et al. 1978). Secondly, most studies of the aquatic chemistry and waste treatability have focused on RDX rather than HMX, since RDX production has far surpassed HMX production. According to Urbanski (1967), HMX has chemical properties very similar to RDX.

Nitramines are substances which contain a nitro group bonded to a nitrogen atom. Nitramines can be considered derivatives of the simplest inorganic nitramine NH_2NO_2. If both hydrogen atoms are replaced by alkyl or aryl groups the compound is termed a secondary nitramine. If only one hydrogen is replaced the compound is a primary nitramine. The two types differ markedly in their chemical and physical properties. RDX and HMX are secondary poly-nitramines in which nitro groups are bonded to the nitrogens of a heterocyclic ring. The process of nitramine formation is referred to as N-nitration. RDX and HMX are completely N-nitrated.

TABLE 1

SUMMARY OF THE GENERAL PROPERTIES OF RDX

Names:	Cyclotrimethylenetrinitramine; Cyclonite; Hexogen; T_4; RDX; Hexahydro-1,3,5-trinitro-1,3,5-triazine, 1,3,5-Trinitrohexahydro-s-Triazine, *sym*-Trimethylenetrinitramine
CAS No.:	121-82-4
Formula:	$C_3H_6N_6O_6$
Molecular Weight:	222.26
Form:	Colorless crystalline solid-orthorhombic crystal structure
Melting Point:	204.1°C
Composition (military grade)	
Carbon:	16.22%
Nitrogen:	37.84%
Oxygen:	43.22%
Hydrogen:	2.72%
Specific Gravity:	1.816
Solubility Properties	
Water:	76 mg/l at 25°C; 1400 mg/l at 83°C
Organic Solvents:	Soluble in aldehydes and ketones
Solvent	
Cyclohexanone:	7.5 g/100 g at 25°C
Cyclopentanone:	9.9 g/100 g at 25°C
Acetone:	8.3 g/100 g at 25°C
Methylacetate:	1.9 g/100 g at 20°C
Nitrobenzene:	1.5 g/100 g at 25°C
Methylisobutenyl Ketone:	3.0 g/100 g at 25°C
Acetic anhydride:	4.9 g/100 ml at 30°C
Slightly soluble in:	Ethanol, ether, benzene, toluene, chloroform, carbon tetrachloride, carbon disulfide, and glycol esters

References: Hawley, 1977; Merck & Co., 1976; Mark, et al., 1965; Small and Rosenblatt, 1974.

TABLE 2

SUMMARY OF THE GENERAL PROPERTIES OF HMX

Names:	Cyclotetramethylenetetranitramine; Octogen; RRI; HMX; Octahydro-1,3,5,7-tetranitroazocine; 1,3,5,7-tetranitro-1,3,5,7-tetraazocyclooctane, Octahydro-1,3,5,7-tetranitro-1,3,5,7-tetrazocine
CAS No.:	2691-41-0
Formula:	$C_4H_8N_8O_8$
Molecular Weight:	296.16
Form:	Colorless crystalline solid, 4 polymorphic forms
Melting Point:	276-280°C
Composition	
Carbon:	16.22%
Nitrogen:	37.84%
Oxygen:	43.22%
Hydrogen:	2.72%
Specific Gravity:	1.87 (β-form)
Solubility Problems	
Water:	6.63 ± 0.35* mg/l at 20°C 11.56 ± 1.92* mg/l at 30°C
Acetone:	2.2 g/100 ml at 30°C
Cyclohexanone:	5.3 g/100 ml at 30°C
Acetic anhydride:	1.3 g/100 ml at 30°C
Generally less soluble in a given solvent than RDX	

*95% confidence limits.

References: Hawley, 1977; Merck & Co., 1976; Mark, et al., 1965; Small and Rosenblatt, 1974; Barkley, Jr., J.J., 1977.

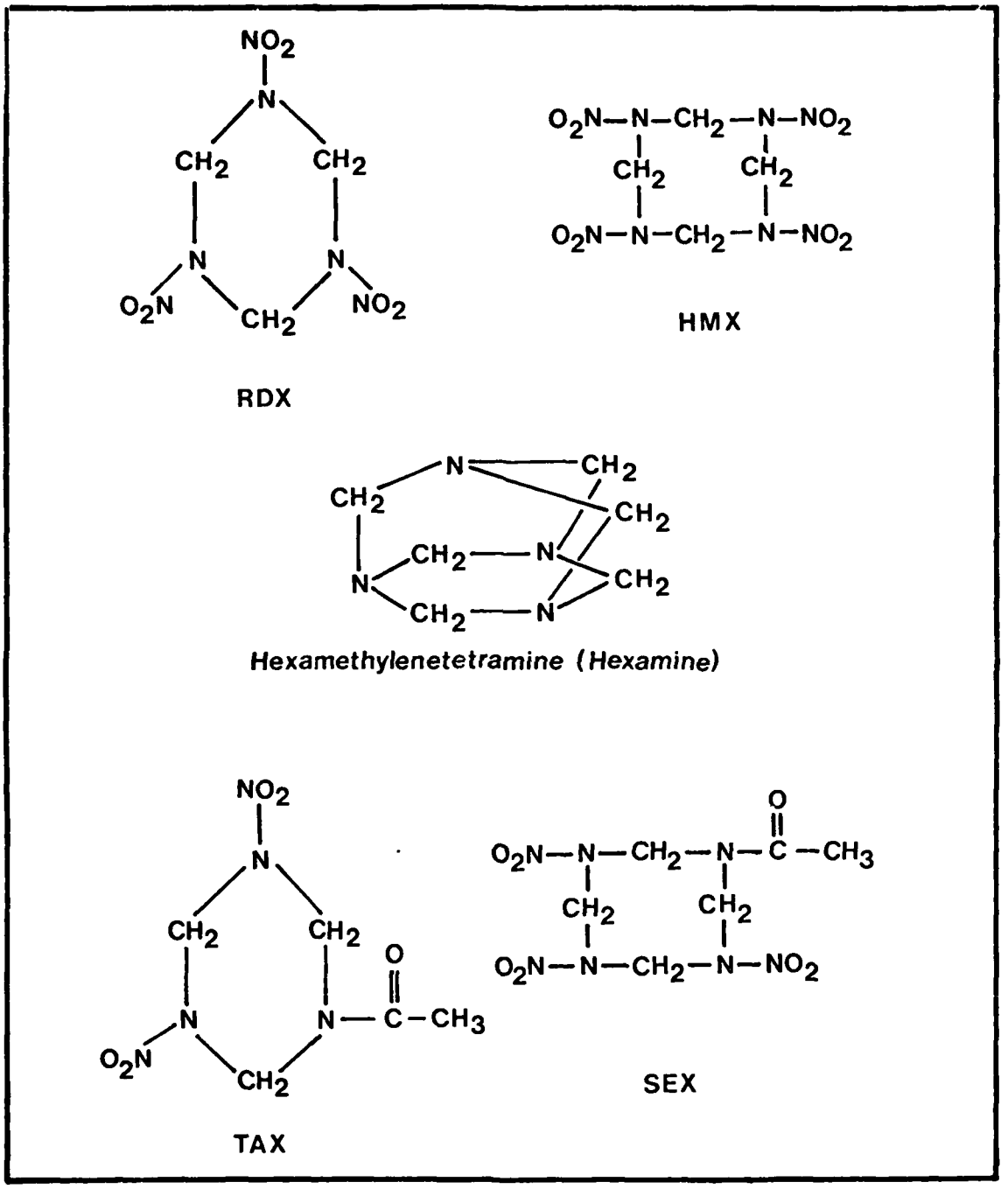


FIGURE 1. MOLECULAR STRUCTURE OF RDX, HMX, AND RELATED COMPOUNDS.
REFERENCE: SMALL AND ROSENBLATT (1974).

RDX is a nitrated 6-membered ring, HMX a nitrated eight-member ring. The theoretical chemistry of nitramines has been reviewed by Urbanski (1967) and Stals (1969A and B).

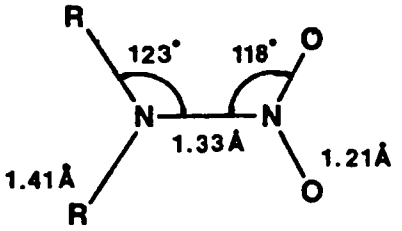
Nitration and commercial preparation of RDX and HMX is effected by means of the nitronium ion (Urbanski, 1967):

$$\text{>N - H} + NO_2^+ \rightleftharpoons \underset{H}{\text{>N}^+} - NO_2 \rightleftharpoons \text{>N - } NO_2 + H^+ \qquad (1)$$

In the Bachmann process (Bachmann and Sheehan, 1949) acetic acid and acetic anhydride facilitate the formation of this ion by polarizing the HNO_3 molecule:

$$O_2\overset{+}{N} - \overset{-}{O}H$$

Urbanski (1967) has given the following model for the planar N-nitrobond. Aromatic and aliphatic bond group dimensions are similar:



(2)

Both the N-N and N-O bonds have partial double bond characteristics (Stals 1969A). In the RDX molecule the N-O bonds are thought to be sp^3 hybrids; in HMX; 2pn hybrids (Stals 1969A).

In RDX and HMX both equatorial and axial NO_2 groups occur. Hydrogen bonding and electrostatic interactions cause bond stresses and force bond assymmetry in the axial NO_2 groups (Stals 1969A and B). Intramolecular hydrogen bonding confers acidic properties on RDX and HMX.

In RDX and HMX crystals, equatorial NO_2 groups pack closely due to favorable intermolecular attraction. This packing forces the axial groups into unfavorable electrostatic positions causing asymmetry (Stals 1969B).

Explosive decomposition of RDX liberates only gaseous products. The products and their ratios reported in the literature differ somewhat. Urbanski (1967) gave the following equation for its decomposition:

$$C_3H_6O_6N_6 \rightarrow 0.93\ CO_2 + 2.01\ CO + 2.13\ H_2O + 0.06\ CH_4 + 3N_2 + 0.72\ H_2 \qquad (3)$$

Urbanski, however, also reported the following composition for the cooled explosion products:

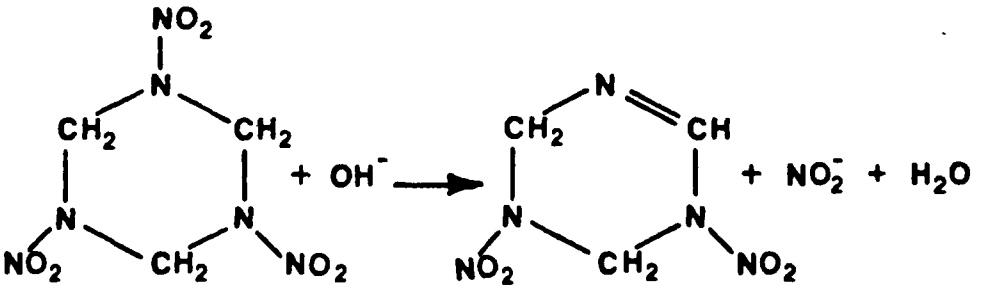
CO_2	19.82 percent	H_2	0.90 percent
CO	25.22 percent	N_2	37.83 percent
H_2O	16.32 percent		

Cook (1958) reported the following products from the explosion of RDX in moles/kg RDX:

CO	0.6	NO	0.1
CO_2	6.6	O_2	0.003
H_2	0.02	OH	0.02
H_2O	3.1	CH_3OH	2.7
N_2	13.0	CH_2O_2	4.0 (formic acid)
NH_3	0.8		

More recently, Stals (1969B) ranked the volatile decomposition products of RDX as follows: in order of decreasing yield: N_2O, CH_2O, N_2, NO, CO_2, HCN, CO. Based on ^{15}N studies, Stals suggested that the NO originates from axial NO_2 groups, that N_2O and N_2 originate from equatorial groups, and that formaldehyde, HCN, CO and CO_2 originate from secondary disintegrations.

Stals theorized that the solvated reactions of RDX and HMX as secondary nitramines are initiated on the carbon atoms by nucleophilic attack (Stals 1969B). The initial products are NO_2^- ions and the alcohols of the nucleophilic agent. Such reactions are thought to occur in stepwise fashion, removing the NO_2 groups, and leaving the skeleton as a heterocyclic aromatic ring. Hoffsommer, et al. (1977) have studied the mechanism of alkaline hydrolysis of RDX and confirmed that the initial attack occurred on the methylene carbon as shown below via proton abstraction. This results in release of nitrite as HNO_2:



(4)

Following this reaction ring opening was found to occur. The final products of hydrolysis included NO_2^-, N_2O, NH_3, N_2, CH_2O and $HCOO^-$. Relative proportions of each depended on the initial OH^- concentration.

RDX decomposes by electrophilic substitution in concentrated H_2SO_4 (Stals 1969B). In general, nitramines are fairly easily decomposed in H_2SO_4, but not NaOH. However, secondary polynitramines such as RDX and HMX are harder to decompose under acid conditions (Urbanski 1967), requiring 100°C and 40 percent H_2SO_4. RDX and HMX, however, are labile under basic hydrolysis.

In terms of explosive strength as well as sensitivity to shock or friction, nitramines occupy a position between nitrate esters and aromatic nitro compounds (Urbanski 1967).

Wastewater Characteristics

Wastewaters containing RDX and HMX result both from the manufacturing and the blending of these compounds with other materials, as well as from the loading of warheads. Manufacturing and blending of RDX and HMX are carried out only at Holston Army Ammunition Plant, Kingsport, Tennessee (HAAP). A second RDX/HMX plant (X-facility) has been proposed, but a final site has not been selected nor construction authorized. Several munitions plants--load, pack, and assembly (LAP) operations - load the various RDX/HMX formulations into bombs and projectiles. Discharges of RDX, HMX, as well as associated compounds resulting from these operations have been reviewed by Small and Rosenblatt (1974), and Patterson et al. (1976), and more recently by Kitchens et al. (1978) Spanggord et al. (1978).

The Manufacturing Process.

RDX and HMX are manufactured at HAAP via, the Bachmann process. This process, reviewed by Kitchens et al. (1978), consists of nitration of hexamine with ammonium nitrate and nitric acid in an acetic acid, acetic anhydride solvent. After nitration, the product is washed to remove spent acids, recrystallized and dried to produce crystals of correct size, then incorporated with such materials as TNT and/or wax to produce the desired explosive formulations.

The processing steps and starting materials are similar for the production of either RDX or HMX. However, the proportions of starting materials differ as follows:

Reactant	lbs. of Reactant per 100 lbs. of Reactant Charge	
	RDX	HMX
Ammonium Nitrate	17.2	6.2
98% Nitric Acid	13.6	4.8
Hexamine	9.2	6.5
Acetic Acid	15.0	28.5
Acetic Anhydride	45.0	54.0

Reported by Small and Rosenblatt (1974).

The stoichiometric reactions for production of RDX and HMX are as follows:

RDX

$$C_6H_{12}N_4 + 4HNO_3 + 2NH_4NO_3 + 6(CH_3CO)_2O \rightarrow 2C_3H_6O_6N_6 + 12CH_3COOH \quad (5)$$

Hexamine	Nitric Acid	Ammonium Nitrate	Acetic Anhydride	RDX	Acetic Acid

HMX

$$2C_6H_{12}N_4 + 8HNO_3 + 4NH_4NO_3 + 6(CH_3CO)_2O \rightarrow 3C_4H_8O_8N_8 + 12CH_3COOH + 6H_2O \quad (6)$$

Hexamine	Nitric Acid	Ammonium Nitrate	Acetic Anhydride	HMX	Acetic Acid

Figure 2 (Kitchens et al. 1978) outlines the flow diagram of the RDX/HMX manufacturing process employed at HAAP as well as the points of entry of the major waste materials discharged to the several process sewers. Most of the RDX and HMX in the HAAP wastewaters is derived from the recrystallization, dewatering and incorporation operations. Monitoring data (see Table 3) suggest that the quantity and quality of effluents from HAAP vary widely, although the overall discharge of munitions has generally decreased over time.

Load, Assemble, and Pack (LAP) Wastewaters.

Loading operations to produce military warheads are carried out at seven army ammunition plants and at four naval facilities:

Army	Navy
Cornhusker (CAAP); Grand Island, Nebraska	Crane; Crane, Indiana
Kansas (KAAP); Parsons, Kansas	Hawthorne; Hawthorne, Nevada
Lone Star (LSAAP); Texarkana, Texas	McAlester; McAlester, Oklahoma
Louisiana (LAAP); Shreveport, Louisiana	Yorktown; Yorktown, Virginia
Milan (MAAP); Milan, Tennessee	
Iowa (IAAP); Middletown, Iowa	
Joliet (JAAP); Joliet, Illinois	

RDX-contaminated wastewater at these facilities is generated from operations such as washdown, melting of explosives, and steam cleaning of rejected warheads. This water contains highly variable quantities of RDX and other materials. Typical LAP wastewater characteristics are presented in Table 4. Spanggord et al. (1978) determined the munitions component ratios in LAP waste in order to determine if a characteristic artificial mixture could be employed in bioassay tests. Forty-seven comparisons of wastewaters from IAAP column influent, MAAP sump water, and LAAP sump water showed a mean TNT/RDX ratio of 1.62. Ratios ranged from 0 to 3.82.

Munitions compounds are removed from LAP wastewaters at IAAP and JAAP by filtration through diatomaceous earth followed by passage through activated carbon columns. Influent and effluent characteristics are

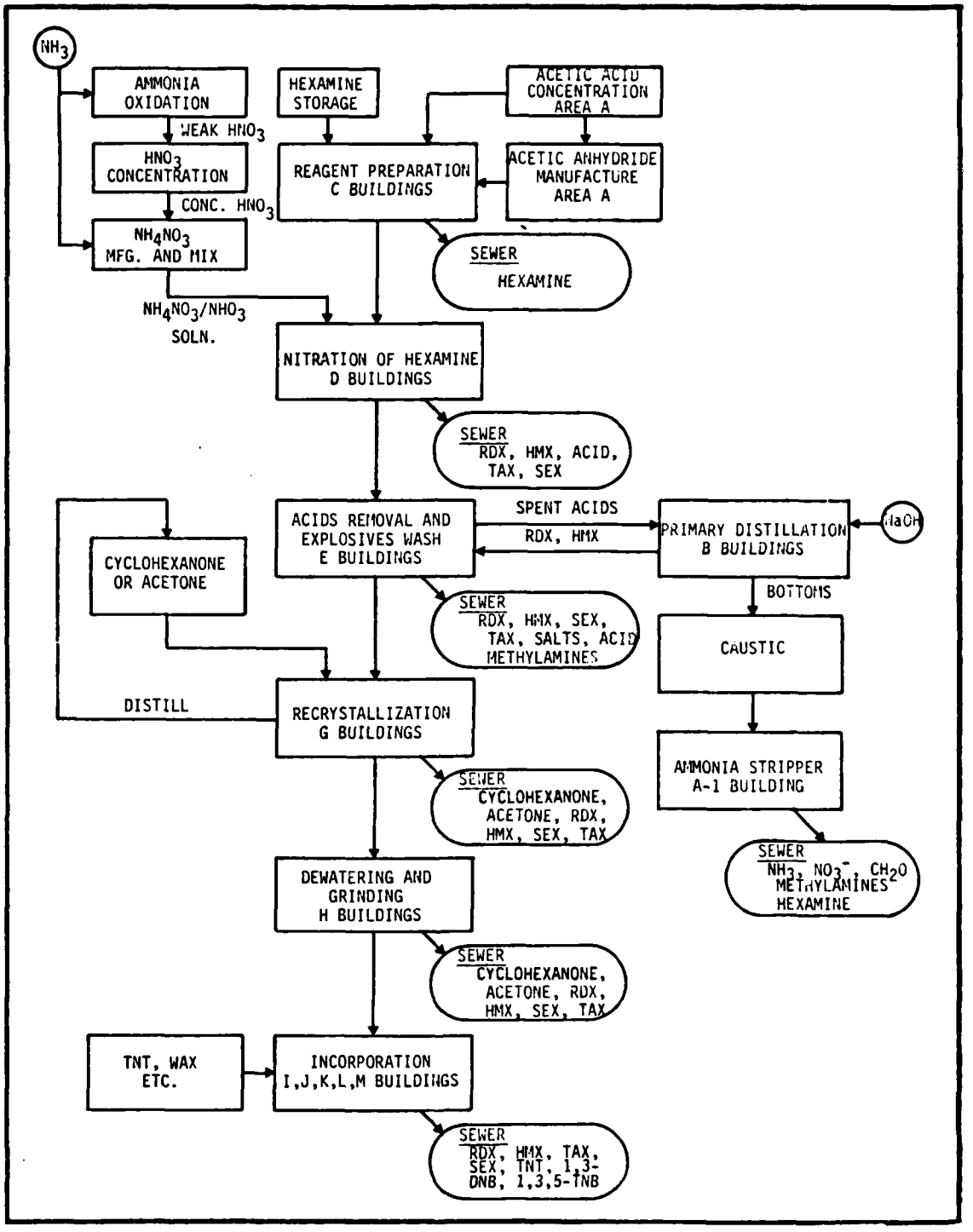


FIGURE 2. FLOW DIAGRAM OF THE RDX/HMX MANUFACTURE AT HAAP. REFERENCE: KITCHENS ET AL. (1978).

TABLE 3

RDX AND HMX CONCENTRATIONS IN HAAP INDUSTRIAL WASTE DISCHARGES 1973-1977

Reference	Waste Source	Period	Number Samples	RDX (mg/l) Mean	RDX (mg/l) Range	HMX (mg/l) Mean	HMX (mg/l) Range	Comments
Patterson et al. (1976)	Area B Process Waste							
	Holston River Mile 139.7	4/2-4/1973	2	0.6	BDL[a]-1.2			Source: EPA 4/1973 Survey
	Holston River Mile 139.6	4/2-4/1973	2	24	15-32			
	Holston River Mile 139.2	4/2-4/1973	2	31	24-38			
	Holston River Mile 139.7	1-6/1973	NR[b]	0.6	BDL-4.3	0.1	BDL-1.4[c]	Source: HAAP Weekly Grab Samples
	Holston River Mile 139.6	1-6/1973	NR	10.4	BDL-24.3	2.6	BDL-8.9	
	Holston River Mile 139.2	1-6/1973	NR	6.5	BDL-12.8	2.1	BDL-7.5	
Sullivan et al. (1977)	Area B Process Waste							
	Prod. Lines 2,3,4,5	6/2-6/1975	5	3.38	BDL-8.78	BDL		$\bar{Q}$ = 21MGD, 48% cooling water
	Prod. Lines 6,7,8	6/2-6/1975	5	2.52	2.19-3.78	BDL		$\bar{Q}$ = 48MGD
	Prod. Lines 2,3,4,5	8/4-8/1975	5	2.02	0.046-8.2	BDL		$\bar{Q}$ = 21MGD, 48% cooling water
	Prod. Lines 6,7,8	8/4-8/1975	5	0.39	BLD-0.9	BDL		$\bar{Q}$ = 48MGD
Stilwell et al. (1977)[c]	Area B	6/2-23/1976	4	0.80	BDL-1.70	0.77	BDL-1.85	
	Area A	6/2-19/1976	8	0.77	BDL-1.90	2.42	0.23-5.40	
Kitchens et al. (1978)	Area B Process Waste							
	Prod. Lines 1-5	1976	25	7.4	BDL-33.9	4.6	BDL-59.3	
	Prod. Lines 6,7	1976	25	5.5	BDL-43.2	2.6	BDL-15.9	
Kitchens et al. (1978)	Area B Process Waste							
	Prod. Lines 1-5	5/17-6/19/1977	7	5.5	1.8-10.4	2.6	0.3-4.7	Source: Holston Defense Corporation
	Blds. D6,E6,G6	5/17-6/19/1977	7	4.5	1.0-7.0	1.6	0.4-2.4	
	Blds. B9,B11,C3,C5,D3, D5,E3,E4	5/17-6/19/1977	7	0.3	0.1-0.4	0.12	BDL-0.6	
Kitchens et al. (1978)	Area B Process Waste							
	Prod. Lines 1-5	1977	9	3.0	0.6-7.6	2.9	0.9-8.4	
	Prod. Lines 6,7	1977	9	1.3	0.1-4.0	1.4	BDL-4.1	

[a]BDL = Below detection limits.

[b]NR = Not reported.

[c]Results reported in ppm.

Prod. = Production
Blds. = Buildings

TABLE 4

TYPICAL MUNITIONS DISCHARGE CONCENTRATIONS FROM LOAD, ASSEMBLE AND PACK OPERATION[a]

Facility	Wastewater Source	Number of Samples	Mean and Range of Constituent Concentration (mg/l) RDX $\bar{x}$	RDX Range	TNT $\bar{x}$	TNT Range	TOC $\bar{x}$	TOC Range	NO_3 $\bar{x}$	NO_3 Range
Iowa (IAAP)	Combined influent to industrial waste treatment plant	24	81	24-170	161	50-260	90	38-138	24	2.6-48
	Effluent-Diatomaceous earth filtration + carbon absorption	23	1.5	<0.1-24	1.3	<0.05-24	5	<1-9	14	3.1-26
Milan (MAAP)	Combined effluent (no treatment except for settling)	30	6.2[b]	0.1-109	3.5[b]	<0.005-210	3.5[b]	<1-122	9.7[b]	4.2-680
Louisiana (LAAP)	Wastewater sump	4	101	34-189	169	78-275	--		137	39-150
	Lagoon system	7	12.3[b]	<0.1-102	3.4[b]	<0.05-44	--		47.3	7.8-110
Lone Star (LSAAP)	Lagoon system (plant inactive)	5	11	<0.1-16	1.3	<0.05-5.4	--		--	

[a]Reference: Spanggord et al. (1978). Data from one year of operation (1976).

[b]Geometric Mean

given for IAAP in Table 4. Table 5 shows the performance characteristics of this type wastewater treatment system at JAAP. The remainder of the Army LAP plants discharge wastewaters into lagoons except for MAAP and the Naval Armaments Depot at Crane, Indiana. MAAP discharges untreated wastewater into ditches leading to the Obion River. The Crane facility discharges untreated LAP wastewater directly onto the ground and into surface streams.

Wastewater Treatment Processes.

Several processes have been evaluated to treat HAAP effluents. Area B wastes, considered the major source of RDX and HMX discharged into the aquatic environment from HAAP, were found to be unsuitable for treatment by activated sludge systems due to formation of filamentous growths. Studies of the effectiveness of three stage biological treatment (dentrification, followed by trickling filter, activated sludge units) showed that the three units operated in a series gave the best munitions removal efficiencies. However, complete removal of nitramine residues was not consistently achieved (Kitchens et al. 1978; Stilwell et al. 1977). Kitchens et al. (1978) concluded that a single stage biological treatment facility would not produce water of sufficient quality to meet 1984 Environmental Protection Agency (EPA) requirements.

Several other tertiary treatment processes have been evaluated on a laboratory or pilot-plant scale for removal of RDX, HMX, and TNT. These processes included anaerobic biodegradation, basic hydrolysis on ion exchange resins, chemical oxidation, coagulation, photolysis using UV light, reverse osmosis, as well as resin and activated carbon adsorption.

Anaerobic Biodegradation. Jackson et al. (1976) also performed treatability studies on RDX and HMX solutions. These authors reported 100 percent removal of RDX and HMX (detection limits not given) by anaerobic fermentation from waters containing 50 mg/l RDX and 15 mg/l HMX in conjunction with supplemental carbon sources; sucrose, methanol and hydroxyethyl cellulose. A mixed cyclohexanone acetone and acetic acid substrate was not able to support nitramine removal. Times required for complete removal of the RDX and HMX are shown in the following table:

Substrate	Time in Days Required for Complete Removal: RDX	HMX
Sucrose	9	7
Methanol	1	5
Hydroxyethyl cellulose	2	7
Hydroxyethyl cellulose in HAAP wastewater	1	7

After essentially complete reduction in HAAP wastewater using hydroxyethylcellulose was achieved, this carbon supplement was removed. Complete nitramine reduction ws maintained for 22 days until the termination of the experiment.

TABLE 5

PERFORMANCE OF LAP WASTEWATER TREATMENT SYSTEMS AT JAAP[a]

Parameter[b]	LAP Influent		Effluent Source:						Average Percent Change
			Diat. Earth Filter		1st Carbon Column		2nd Carbon Column		
	Range	Average	Range	Average	Range	Average	Range	Average	
pH	6.8-8.4	7.9	7.6-8.6	7.9	7.6-8.2	7.8	6.7-8.0	7.7	-
Total Solids	903-1790	1401.5	959-1796	1418.5	794-1791	1138.3	762-1497	1069.8	23.7
Suspended Solids	220-336	138.5	26.0-271.0	108.6	0.0-40.0	8.4	0.0-7.0	1.2	99.1
TOC	93-188.4	121.1	100.0-162.0	121.1	5.9-64.0	24.3	7.8-20.9	12.1	90.0
Kjeldahl-N	10.3-25.4	17.0	8.9-23.0	15.3	4.4-8.6	7.2	4.0-4.9	4.6	72.9
TNT	156.2-235.0	178.2	143.5-213.0	175.7	0.0-111.2	14.7	0.0-25.0	3.7	97.9
RDX	87.5-180.0	145.2	87.5-185.0	148.9	0.0-77.5	30.1	0.0-46.4	19.5	86.6
Color	-	-	-	-	5.0-20.0	10.0	0.0-20.0	8.0	-

[a]Reference: Patterson et al. (1976).

[b]All units except pH and color in mg/l. Color in Platinum-Cobalt Units (PCU)

Basic Hydrolysis. Small and Rosenblatt (1974) have reviewed the earlier literature regarding methods for decomposing HMX and RDX and report that boiling with 5 percent NaOH will destroy both HMX and RDX. NH_3, CH_2O, NO_2^-, and NO_3^- are liberated. HMX is apparently more resistant to basic hydrolysis than RDX. Dilute sulfuric acid also decomposes RDX to N_2 and CH_2O or NH_3, N_2O, and CH_2O; depending on the acid strength. Acid or alkaline hydrolysis of dilute solutions of RDX or HMX is impractical, however, since the amount of acid or base required for treatment and subsequent neutralization would be large. Hoffsommer and Kubose (1977) have studied the kinetics of basic hydrolysis and suggest that concentration and destruction of RDX by strongly basic ion exchange resins was a potential single-step waste treatment method. In this approach aqueous solutions of RDX were adsorbed by the resin and reacted with quaternary ammonium hydroxide. The ion exchange resin was found to be easily regenerated by conversion first to the chloride form then to the hydroxide. This procedure, however, is only feasible for aqueous solutions which have fairly low acid and/or anion concentrations. Most munitions wastewaters contain high concentrations of waste acids or neutralized salts.

Chemical Oxidation. Chemical oxidation using potassium dichromate, potassium permanganate, or calcium hypochlorite will destroy nitramines. Jackson et al. (1976) found that 1,000 mg $Ca(OCl)_2$, $K_2Cr_2O_7$, and $KMNO_4$ completely oxidized 50 mg/l RDX within 72, 48, and 24 hours respectively. However, 1000 mg/l of the oxidants were required. Oxidation products were not quantitated. Ozonation apparently is not effective for RDX and HMX oxidation (Jain 1976).

Coagulation. Chemical flocculation and coagulation is one process which may be employed to remove dissolved and suspended solids from a munitions waste stream. Jackson et al. (1976) performed preliminary experiments on the removal of RDX and HMX by chemical coagulation using lime, ferric chloride, and Cat-Floc-T, a cationic polymer. Ninety percent reduction was achieved using a 500 mg/l lime dose. Castorina et al. (1977) have cautioned against use of Cat-Floc-T, however, since it is not compatible with RDX and TNT in a dry form and sludge dewatering might cause adverse reactions. Only E-653 was found to be compatible; WT-2600 and Cat-Floc, the other polymers tested, also reacted with the explosives.

Ultraviolet Light Photolysis. Kubose and Hoffsommer (1977) have shown that concentrations of 20 to 40 mg/l RDX in aqueous solution could be reduced by 98 percent by photolysis using the full spectral output from a medium pressure mercury vapor lamp (220nm-1367nm) with irradiation periods of 15 seconds in a laboratory column flow system. Andrews and Osmon (1977) have also irradiated aqueous solutions of RDX and HMX as well as wastewater containing both TNT and RDX. The nitramine compounds were amenable to such treatment. Complete photolysis of 45.5 mg/l RDX and 5.6 mg/l HMX was accomplished within 2 hours. The chemistry of photolysis will be discussed in a later section of this report. No breakdown products were detected after 2 hours irradiation using thin layer chromatographic techniques. Jain (1977) used UV irradiation in adsorption-oxidation studies of TNT and RDX degradation. This investigator attributed successful oxidation of adsorbed RDX by ozone to UV

pretreatment. The UV light itself may have been the element responsible for the enhanced RDX removal of the adsorbed solution rather than the oxidant. Ultraviolet light is effective as a treatment method. High treatment costs would be expected in order to generate the energy for producing UV light.

Reverse Osmosis. Reverse osmosis was tested on HAAP Area B wastewater by Jackson et al. (1976). RDX, HMX, and TNT breakthrough occurred within 24 hours. This implies that reverse osmosis is not effective in treating RDX and HMX wastewaters.

Adsorption. Jackson et al. (1976) also studied RDX, HMX, and TNT removal, using activated carbon and Rohm and Haas XAD-2 resins. Both completely removed the munition compounds from HAAP wastewater (detection limits not given). Activated carbon however, was superior to resin at a hydraulic loading of 10 gpm/ft^3.

Adsorbant	Hours to Breakthrough		
	RDX	HMX	TNT
Carbon	176	176	Did not
XAD-2	48	16	break through
Concentration Range during test (mg/l)	1.5-12	0-4.0	0-2.0

Vlahakis (1974) and Szachta (1978) have also studied adsorption of munitions. Vlahakis evaluated activated carbon, reverse osmosis, ion exchange, polymeric adsorption, hydrolysis, boiling, and chlorination as treatments to remove RDX from contaminated groundwater. Carbon adsorption proved to be the most satisfactory technique in laboratory scale tests. This author found that TNT inhibited RDX adsorption. In the presence of TNT activated carbon had a capacity of 76 mg RDX/g carbon as opposed to 125 mg RDX/g carbon for RDX alone.

Szachta (1978) evaluated laboratory, pilot plant, and full scale plant tests of carbon and amberlite-XAD-4 adsorption to treat pink water from load, pack, and assemble facilities. Amberlite resin had a higher capacity for TNT than carbon. Carbon, however, had a higher capacity for RDX and HMX. Szachta recommended activated carbon with regeneration as the most effective treatment method.

III. Analytical Methods/Environmental Monitoring

The techniques available for quantitative analysis of RDX and HMX include volumetric analysis, thin layer chromatography, high pressure liquid chromatography, gas-liquid chromatography, and single-sweep polarography. Volumetric techniques, summarized in Table 6, have been used to determine the proportions of RDX and HMX in solid munitions samples and have not been modified to detect the low levels expected in samples from the aquatic environment. A rapid, quantitative assay for RDX and HMX at concentrations of 0.05 mg/l or more in water using polarography has been developed by U.S. Navy researchers (Prestia 1975; and Whitnack 1976). Most workers have employed either gas liquid chromatography (glc) plus thin layer chromatography (tlc), or high pressure liquid chromatography (hplc) to routinely measure low levels RDX and HMX in environmental

TABLE 6

VOLUMETRIC METHODS FOR ANALYSIS OF RDX AND HMX[a]

METHOD	SOLVENT	REAGENT	TITRANT	SENSITIVITY
Reduction	dimethylformamide	chromous chloride	ferric ammonium sulfate	± 0.02 mg
Hydrolysis	sulfuric acid		ferrous ammonium sulfate	± 2.0 mg
Acid-Base	methylisobutyl ketone-isopropanol, 4:1		sodium methoxide	± 0.2 mg
Acid-Base	methyl isobutyl ketone		tetrabutylammonium hydroxide	± 0.2 mg
Acid-Base	dimethylformamide		sodium methoxide	± 0.4 mg

[a]Reference: Small and Rosenblatt (1974)

samples. Because of the low vapor pressures and thermal instability of HMX, gas chromatography cannot be used to detect this compound at low levels (Stilwell et al. 1977). Because of the ease of photolysis of RDX and HMX, samples must be stored in the dark in amber glass bottles with teflon-lined stoppers. Most researchers have also stored samples at 4°C (Sullivan et al. 1977; Spanggord et al. 1978; and Stilwell et al. 1977). Where significant suspended solids were present Stilwell et al. (1978) recommended that samples be filtered and the suspended material washed with tetrahydrofuran. These washings can then be combined with the filtrate for analysis.

Polarography. Prestia (1975) suggested that polarography using a dropping mercury electrode system could be used to quantitate RDX and HMX in mixtures of munitions compounds or environmental samples, based on their different and reproducible peak potentials. Whitnack (1976) developed a technique (single-sweep polarography) which could detect 0.05 mg/l RDX or HMX in a 2 ml aliquot of water or effluent sample. According to Whitnack, analysis time was about 5 minutes and reproducibility ± 10 percent.

In this analytical technique samples are either acidified with HCl or a neutral electrolyte (usually NaCl) is added. Dissolved oxygen is purged from the system using nitrogen gas. The change in current is measured at potentials ranging from -0.1 to -0.8 volts. RDX produces a unique peak at -0.48 volts, HMX at -0.58 volts. TNT or DNT interfere with detection in acidified solutions. If these compounds are present, resolution of RDX and HMX is carried out under neutral conditions. Selection of an electrolyte depends on the overall constituency of the sample and is determined by preliminary experiments. Metal ions and certain anions interfere with RDX and HMX detection. The extent of these interferences has not been completely tested. Whitnack (1976) recommends EDTA addition to prevent cation interference. Anions must be oxidized to eliminate their interference.

Thin Layer Chromatography. Thin layer chromatography on silica gel plates can be used to detect RDX and HMX in waters and sediments. Leach and Hash (1972) reported detection limits of 25 mg/l RDX and 40 mg/l HMX for direct determination. Sample evaporation could be used to lower the effective detection limit to 1 mg/l of each compound. Sullivan et al. (1977) reported detection limits for HMX of 1 ppm for sediment or water samples. Ethyl acetate was used to chromatograph HMX which was detected by UV light (254 nm). Ethylenediamine in dimethyl sulfoxide was used to enhance resolution. Jain (1976) used tlc to quantitate HMX from wastewater used in absorption experiments. However, he used mass spectrometric methods to determine HMX content. His detection limit was 0.2 mg/l. RDX is not generally quantitated by tlc since lower detection limits are possible for this compound using the following two methods.

Other researchers have employed tlc extensively to separate munitions components such as RDX, TNT, HMX and photolysis products from extracts for qualitative identifications (Hale et al. 1978; Stilwell et al. 1977; Jackson et al. 1976).

Gas-Liquid Chromatography. Several workers have routinely used glc techniques to measure RDX in water, sediment, and soil samples contaminated with mixtures of munitions residues. Electron capture (^{63}Ni), flame ionization, and alkaline earth flame ionic detection systems provide resolution of RDX from TNT and other materials in the parts per billion range. There are two major drawbacks to the use of gas chromatography for nitramine detection. First, only RDX can be detected; HMX is not sufficiently volatile to be chromatographed in this manner. Secondly, sample preparation is somewhat complicated and time consuming, and entails a risk of sample loss or destruction. Table 7 summarizes the detection systems and limits for gc analysis of RDX.

High Peformance Liquid Chromatography. This technique overcomes the problems of thermal instability and low volatility which hinder gc analysis of RDX and HMX. Analysis time for hplc is rapid; a sample can be analyzed in approximately 15 minutes (Stilwell et al. 1978). This procedure is not as sensitive for RDX as glc analysis, but provides a reasonably sensitive test for HMX. Stilwell et al. (1978) was able to detect as little as 0.05 mg/l RDX or HMX. However, below 0.1 mg/l reproducibility was ± 50 percent. Ultraviolet light absorption is generally used for detection. A variable wave length detector can be used to enhance sensitivity by selecting a wave length that maximizes the absorbances for the materials of interest. The detection limits and analytical systems employed for hplc of RDX and HMX are shown in Table 8. Using careful analytical techniques, hplc probably affords the best analytical system for routine monitoring of trace concentrations of RDX and HMX in the aquatic environment.

IV. Toxicological Aspects

Bentley et al. (1978), under contract with the U.S. Army Medical Research and Development Command, performed an extensive laboratory evaluation of the acute toxicity of RDX to a wide variety of aquatic organisms representing four important trophic levels: algal primary producers, zooplankton, invertebrates and fish. More definitive chronic studies, including embryo/larvae and life-cycle tests, as well as an evaluation of the bioconcentration potential of RDX were performed using selected test organisms. Liu et al. (1977) examined the toxicity of wastewaters and wastewater constituents (including RDX) associated with the manufacture and processing of TNT to aquatic life. The acute toxicity of RDX to two species of freshwater organisms was investigated as well as the interaction between RDX and TNT and its photolysis products.

The data base available for the hazard evaluation of HMX in the aquatic environment is somewhat more restricted than for RDX. Bentley et al. (1977) performed a laboratory evaluation of the acute toxicity of HMX utilizing the same test organisms employed with RDX. This work comprises the entire data base for the hazard evaluation of HMX in the aquatic environment.

RDX Toxicology

Freshwater Algae. Bentley et al. (1978) investigated the toxicity of RDX to four species of freshwater algae. Two of the species were

TABLE 7

SUMMARY OF GAS CHROMATOGRAPHIC ANALYTICAL CONDITIONS FOR RDX DETECTION

Reference	Extraction Solvent	Detection Method[a]	Carrier Gas	Detection Limit(ppm)
Hoffsommer et al. (1977)	Benzene Acetone	EC,(^{63}Ni)	Argon/Methane; 95/5	0.002[b]
Jain (1976)	Benzene (Conc. by vacuum evaporation of acetone)	FID and EC(^{63}Ni)	Helium	0.03
Sullivan et al. (1977)	Ethylacetate	AFID or EC	Nitrogen	0.005 water 0.2 sediment
Bentley et al. (1978)	Benzene	EC(^{63}Ni)	Nitrogen	Not given

[a] Detections: EC = Electron capture
FID = Flame ionization
AFID = Alkaline earth, flame ionization (Thermionic)

[b] Personal Communication, J.C. Hoffsommer.

TABLE 8

SUMMARY OF HIGH PERFORMANCE LIQUID CHROMATOGRAPHIC ANALYTICAL CONDITIONS FOR RDX/HMX DETECTION

References	Material Assayed	Extracting Agent	Column Mobile Phase	Column Stationary Phase	Detector Wavelength nm	Detection Limit (mg/l or ppm) RDX	HMX
Hale et al. (1978)	Soil	Ether	30% CH_3OH/H_2O	Zorbax-ODS	230	0.05	---
	Water	Ether	30% CH_3OH/H_2O		230	0.05	---
Spanggord et al. (1978)	LAP H_2O	---	60/40 CH_3OH/H_2O	M-Bondapak C_{18}	254 210	0.4 0.2	--- ---
Stilwell et al. (1977)	HAAP/ Waste-water	Ethyl Acetate	40/60 CH_3OH/H_2O	Partisil 10-ODS	230	0.05	0.05
Jackson et al. (1976)	Aq. RDX/HMX	CH_2Cl_2/ acetonitrile 85/15	CH_3OH 5 Acetonitrile 10 $CHCl_3$ 15 Hexane 70	μ-Partisil	254	---	---

eucaryotes: Navicula pelliculosa, a diatom; and Selenastrum capricornutum, a chlorophyte. The other two species, Microcystis aeruginosa and Anabaena flos-aquae, were members of the procaryotic Myxophyceae. Procedures outlined by the EPA (Algal Assay Procedure: Bottle Test, EPA 1971) were used to define the response to five nominal RDX concentrations ranging from 0.32 to 32.0 mg/l plus control. Chlorophyll a and either cell numbers (N. pelliculosa. S. capricornutum and M. aeruginosa) or optical density (A. flos-aquae) measurements were obtained from triplicate exposed cultures.

Bentley et al. (1978) reported 96-hour EC50's greater than 32 mg/l for all four algae species tested. However, their use of the probit transformation to analyze growth response relative to a control is a questionable procedure (Finney 1971). In order to estimate a no-effect concentration, a one-way analysis of variance (ANOVA) was performed on the 96-hour chlorophyll a and cell count (or optical density) data of Bentley et al. (1978). Subsequently, in tests where a significant ($P < 0.05$) response was indicated, a one-sided Dunnett's test (Dunnett 1955) as well as Williams' procedure (Williams 1971) for comparison of individual treatment means to control were applied to the data. Table 9 summarizes the results of this analysis.

S. capricornutum was the most sensitive of the four algae species tested. RDX significantly ($P \leq 0.05$) inhibited the growth of all four algae species after 96 hours exposure using cell counts (or optical density) as the measure of growth response. S. capricornutum exhibited a slight (less than 2 percent), but significant ($P \leq 0.05$), reduction in mean cell counts at the lowest dose level, 0.32 mg/l.

S. capricornutum was the only algae species tested to exhibit a significant reduction in chlorophyll a after 96 hours exposure to RDX. The effect, although slight, (3 percent) was statistically significant using the Williams test at the lowest challenge concentration of 0.32 mg/l. This precludes the determination of a no-effect level for this species.

The extreme sensitivity of the statistical methods employed in resolving the response level of S. capricornutum is due to the very low variance of the measurements obtained from the replicated cultures. In this case the biological significance of 2 and 3 percent charges that were found to be statistically significant might be questioned.

Freshwater Invertebrates. Bentley et al. (1978) conducted flow-through and static, acute bioassays using two species of freshwater invertebrates: the midge Chironomus tentans and Daphnia magna, a Cladoceran (see Table 10 and 11). Static, acute tests were also conducted by these investigators using the amphipod Gammarus fasciatus and the isopod Asellus militaris. Liu et al. (1977) also conducted a static, acute test using D. magna as the test organism and RDX challenge concentrations as high as 41 mg/l. As a group, invertebrates appear to be far less sensitive than either fish or algae to the acute effects of RDX. After 48 hours in both static and flow-through bioassays no adverse effects attributable to RDX toxicity were reported for any species tested, even at the highest challenge concentrations. Thus, only a lower

TABLE 9

RESPONSE OF FOUR FRESHWATER ALGAE SPECIES TO RDX

Algae Species Tested	Lowest Significant ($P \leq .05$) Response (Chlorophyll a) Concentration at 96 Hours		Lowest Significant ($P \leq .05$) Response (Cell Counts[b]) Concentration at 96 Hours	
	Dunnetts[a]	Williams	Dunnetts[a]	Williams
Eucaryotes:				
Naviculla pelliculosa	N.S.[c]	N.S.[c]	10.0 mg/l	10.0 mg/l
Selenastrum capricornutum	1.0 mg/l	0.32[d] mg/l	0.32[d] mg/l	0.32[d] mg/l
Procaryotes:				
Microcystis aeruginosa	N.S.[c]	N.S.[c]	32.0 mg/l	32.0 mg/l
Anabaena flos-aquae	N.S.[c]	N.S.[c]	10.0 mg/l	10.0 mg/l

[a]One Tail Test

[b]Response was determined by cell counts for all species except A. flos-aquae in which the response was determined by optical density.

[c]Not Significant ($P \leq 0.05$)

[d]Lowest challenge concentration

TABLE 10

ACUTE TOXICITY OF RDX TO FRESHWATER INVERTEBRATES
UNDER STATIC TEST CONDITIONS

Organism	Acute Toxic Concentration (mg RDX/l) [a,b]		Reference
	48-Hour EC50	Other	
Freshwater Invertebrates			
Cladoceran *Daphnia magna*	>100 >41	24-hr EC50, >100	Bentley *et al.* (1978) Liu *et al.* (1977)
Amphipod *Gammarus fasciatus*	>100	24-hr EC50, >100	Bentley *et al.* (1978)
Isopod *Asellus militaris*	>100	24-hr EC50, >100	Bentley *et al.* (1978)
Midge *Chironomus tentans*	>100	24-hr EC50, >100	Bentley *et al.* (1978)

[a]EC50 based on immobilization of organism.

[b]Unless otherwise noted, all reported values based on nominal concentrations.

TABLE 11

ACUTE TOXICITY OF RDX TO FRESHWATER INVERTEBRATES
UNDER FLOW-THROUGH TEST CONDITIONS

Organism	Acute Toxic Concentration (mg RDX/l)[a,b]		Reference
	48-Hour EC50	Other	
Freshwater Invertebrates			
Cladoceran *Daphnia magna*	>15	24-hr EC50, >15	Bentley *et al.* (1978)
Midge *Chironomus tentans*	>15	24-hr EC50, >15	Bentley *et al.* (1978)

[a]EC50 based on immobilization of organism.

[b]Unless otherwise noted, all reported values based on nominal concentrations.

limit based on nominal RDX concentrations can be placed on the 48-hour EC50's for the species tested, i.e., greater than 100 mg/l for the static tests and greater than 15 mg/l for the flow-through tests.

Liu et al. (1977) performed a series of static and flow-through tests designed to characterize the interaction between RDX and TNT and its photolysis products on the acute toxicity of these constituents to D. magna. However, no definitive conclusions could be derived from the results presented.

A chronic study was conducted by Bentley et al. (1978) using D. magna as the test organism (see Table 12). This bioassay was performed under flow-through conditions with mean measured RDX concentrations ranging from 1.4 to 20 mg/l. In the first generation, the total number of young per treatment as well as the number of young produced per parthenogenic female were significantly ($P \leq 0.05$) reduced with respect to controls after 14 days exposure to RDX concentrations as low as 9.5 and 4.8 mg/l, respectively.

A chronic study using C. tentans as the test organism, flow-through conditions following an initial 2-day static early instar period, and measured RDX concentrations ranging from 1.3 to 21 mg/l was also performed by Bentley et al. (1978). In the first generation, no significant effect on larvae, pupae or adult survival or adult emergence was observed, except for complete larvae mortality in one replicate at 10 mg/l RDX on day 14. However, these investigators suspected an equipment malfunction. A significant reduction in second generation adult emergence was observed at 2.2 mg/l RDX, on larvae survival at all RDX levels, and on adult survival at 1.3 and 4.0 mg/l RDX. However, many of these effects were not observed at higher RDX concentrations and interpretation of these results was further complicated by the fact that the second generation at 1.3, 4.0 and 10.0 mg/l RDX was initiated from control eggs.

Freshwater Fish. Based on work by Bentley et al. (1978) and Liu et al. (1977), fish appear to be the most sensitive group of freshwater organisms to the acute toxicity of RDX. Bentley et al. (1978) conducted flow-through and static, acute bioassays of RDX using three freshwater fish species: bluegill, Lepomis macrochirus; channel catfish, Ictalurus punctatus; and fathead minnow, Pimephales promelas. Static, acute tests were also conducted by these investigators using rainbow trout, Salmo gairdneri. Liu et al. (1978) also conducted a static, acute bioassay of RDX using a P. promelas as the test organism. Chronic studies of the effects of RDX were carried out by Bentley et al. (1978) in partial life-cycle tests using channel catfish and fathead minnows as test organisms. A complete life-cycle test was also performed, using the fathead minnow as test organism. All static and flow-through acute bioassays reported by Bentley et al. (1978) were based on nominal RDX concentrations. Partial life-cycle, i.e., 30 day egg-fry, tests carried out by Bentley et al. (1978) utilized measured concentrations for three of five treatments with the remaining two concentrations estimated by interpolation on a logarithmic scale. Full life-cycle tests utilized measured RDX concentations at all treatment levels.

TABLE 12

LONG TERM RDX TEST RESULTS FOR FRESHWATER INVERTEBRATES

Organism	Test Description	Variable	Lowest Significant (P<0.05) Response Concentration (mg RDX/l)	
			Dunnett[a]	Williams
Freshwater Invertebrates				
Daphnia magna	Full Life Cycle	1st Generation - 14 Days Total Number Young	9.5	9.5
		1st Generation - 14 Days Number Young Per Female	4.8	4.8

[a]One Tail Test

RDX exerted an acute, toxic effect on all freshwater fish species tested at concentrations ranging from 3.6 to 6.4 mg/l. Tables 13 and 14 summarize the acute toxic responses found under static and flow-through test conditions, respectively. The bluegill, used to test the effects of changes in temperature, pH and water hardness, was the most sensitive freshwater fish to the acute effects of RDX, having a minimum 96-hour LC50 of 3.6 mg/l at 20°C, pH 6.0 and 35 mg $CaCO_3$/l total hardness. However, none of these water quality parameters, in the ranges tested, significantly affected the sensitivity of the bluegill to RDX.

The acute toxicity of RDX to various life stages was examined under static test conditions by Bentley et al. (1978) using the fathead minnow as the test organisms. Eggs were the most resistant life stage tested. Susceptibility of this organism was greatest at 7 days post-hatch.

In static, acute bioassays, the 96-hour LC50 of RDX in test solutions aged up to 96 hours prior to the introduction to the test organisms, i.e., bluegill, remained essentially constant with a coefficient of variation of less than 3 percent. Bluegill and fathead minnows exposed to RDX under flow-through conditions exhibited 96-hour LC50's similar to those observed under static conditions. Channel catfish, however, exhibited increased resistance to RDX under flow-through conditions. The incipient (i.e., 264-hour) LC50's of these three fish species were only slightly lower, 15-21 percent, than their respective 96-hour LC50's.

Liu et al. (1977) peformed a series of static, acute, bioassays designed to characterize the interaction between RDX and TNT and its photolysis products on the acute toxicity of these constituents to the fathead minnow. The results of these tests failed to demonstrate a synergistic effect at RDX:TNT ratios of from 1:0.33 to 1:3.

The results from the longer term tests were originally evaluated statistically by means of the analysis of variance (ANOVA) followed by Dunnett's method of pairwise comparison of individual treatments means with control. Unfortunately, a number of these analyses were made utilizing average replicate values within the various tests. In order to obtain the maximum amount of information from the data, statistical analyses were repeated utilizing individual, raw data values pooling controls when appropriate and taking into account the blocked experimental design. In addition, Williams' procedure (1972) as well as the one-sided Dunnett's test, was applied to the data. Williams' procedure is specifically designed to detect differences in situations where an increasing or decreasing response with challenge concentrations is expected (see Table 15).

The 30-day partial life-cycle test using channel catfish, performed by Bentley et al. (1978), was beset by an equipment malfunction causing the results of this study to be suspect. A similar study performed by these investigators using fathead minnows indicated no significant reduction in either hatchability or survival of fry after 30 days exposure to RDX concentrations as high as 5.8 mg/l. However, a slight, 5.6 percent, but significant ($P < 0.05$) reduction in mean length of fry exposed continuously for 30 days to RDX concentrations as low as 3.0 mg/l was observed.

TABLE 13

ACUTE TOXICITY OF RDX TO FRESHWATER FISH
UNDER STATIC TEST CONDITIONS

Organism	Acute Toxic Concentration (mg RDX/l)[a]		Comments[b]	Reference
	96-Hour LC50	Other		
Freshwater Fish				
Bluegill *Lepomis macrochirus*		24-hr LC50, 14(12-17) 48-hr LC50, 8.5(7.5-9.5)		Bentley *et al.* (1978)
	6.0(5.4-6.5)			
	8.4(6.0-11)		T=15°C, pH=7.0, TH=35 mg/l $CaCO_3$	
	5.1(3.9-6.7)		T=20°C, pH=7.0, TH=35 mg/l $CaCO_3$	
	4.1(3.0-5.6)		T=25°C, pH=7.0, TH=35 mg/l $CaCO_3$	
	3.8(2.0-7.1)		T=20°C, pH=7.0, TH=35 mg/l $CaCO_3$	
	5.3(4.1-6.8)		T=20°C, pH=7.0, TH=100 mg/l $CaCO_3$	
	3.9(2.1-7.3)		T=20°C, pH=7.0, TH=250 mg/l $CaCO_3$	
	3.6(1.9-6.6)		T=20°C, pH=6.0, TH=35 mg/l $CaCO_3$	
	3.7(2.0-6.9)		T=20°C, pH=7.0, TH=35 mg/l $CaCO_3$	
	3.9(2.1-7.3)		T=20°C, pH=8.0, TH=35 mg/l $CaCO_3$	
	4.7(3.4-6.5)		Initial Age of RDX Test Solution = 0 hr	
	4.8(3.5-6.8)		Initial Age of RDX Test Solution = 12 hrs	
	5.1(3.6-7.2)		Initial Age of RDX Test Solution = 24 hrs	
	4.8(3.5-6.7)		Initial Age of RDX Test Solution = 48 hrs	
	4.8(3.5-6.7)		Initial Age of RDX Test Solution = 96 hrs	
Fathead Minnow *Pimephales promelas*		24-hr LC50, 10(7.4-14) 48-hr LC50, 8.4(6.4-11)[c]		Bentley *et al.* (1978)
	5.8(4.7-7.2)			
		24-hr LC50, >100	Eggs	
		48-hr LC50, >100	Eggs	
	>100		Eggs	
		24-hr LC50, >100	1 hr, Post Hatch, Fry	
		48-hr LC50, >100	1 hr, Post Hatch, Fry	
	43(27-69)		1 hr, Post Hatch, Fry	
		24-hr LC50, >32	7 Days, Post Hatch, Fry	
		48-hr LC50, 18(13-24)	7 Days, Post Hatch, Fry	
	3.8(3.0-5.0)		7 Days, Post Hatch, Fry	
		24-hr LC50, 18(13-24)	30 Days, Post Hatch, Fry	
		48-hr LC50, 16(13-19)	30 Days, Post Hatch, Fry	
	16(13-19)		30 Days, Post Hatch, Fry	
		24-hr LC50, 11(6.1-21)	60 Days, Post Hatch, Fry	
		48-hr LC50, 11(5.9-21)	60 Days, Post Hatch, Fry	
	11(5.9-21)		60 Days, Post Hatch, Fry	
		24-hr LC50, 6.2(5.3-7.3)		Liu *et al.* (1977)
	5.3(4.3-6.5)			

Continued on following page.

Table 13 (continued)

Channel Catfish *Ictalurus punctatus*	4.1(3.5-4.9)	24-hr LC50, 7.5(6.7-8.5) 48-hr LC50, 6.0(5.3-6.9)		Bentley *et al.* (1978)
Rainbow Trout *Salmo gairdneri*	6.4(5.4-7.4)	24-hr LC50, 9.4(8.5-10) 48-hr LC50, 7.0(6.3-7.7)		Bentley *et al.* (1978)

[a]Numbers in parentheses represent 95 percent fiducial/confidence limits

[b]Unless otherwise noted, all reported values based on nominal concentrations

[c]Recalculated values

TABLE 14

ACUTE TOXICITY OF RDX TO FRESHWATER FISH
UNDER FLOW-THROUGH TEST CONDITIONS

Organism	Acute Toxic Concentration (mg RDX/l) [a]		Comments [b]	Reference
	96-Hour LC50	Other		
Freshwater Fish				
Bluegill Lepomis macrochirus	7.6(5.6-10)	24-hr LC50, >10 264-hr LC50, 6.4(5.3-7.8)	 Incipient LC50	Bentley et al. (1978)
Fathead Minnow Pimephales promelas	6.6(5.0-8.7)	24-hr LC50, >10 264-hr LC50, 5.2(4.3-6.4)	 Incipient LC50	Bentley et al. (1978)
Channel Catfish Icatalurus punctatus	13(8.8-20)	24-hr LC50, >10 264-hr LC50, 11(9.1-13)	 Incipient LC50	Bentley et al. (1978)

[a] Number in parentheses represent 95 percent fiducial/confidence limits.

[b] Unless otherwise noted, all reported values based on nominal concentrations.

TABLE 15

LONG TERM RDX TEST RESULTS FOR FRESHWATER FISH

Organism	Test Description	Variable	Lowest Significant ($P \leq 0.05$) Response Concentration (mg RDX/l)	
			Dunnett[a]	Williams
Channel Catfish	Embryo-Larvae	30-Day % Survival	1.2[b]	1.2[b]
Fathead Minnow	Embryo-Larvae	30-Day Length	3.0	3.0
	Full Life-Cycle Test 1	60-Day % Survival	4.9	4.9
	Full Life-Cycle Test 2	30-Day % Survival	6.3	6.3
		60-Day %Survival	6.3	6.3
		Spawns Per Female	6.3[c]	
		Eggs Per Female	6.3[c]	

[a]One-tailed test.

[b]Equipment malfunction suspected.

[c]Increased response with respect to control.

Two life-cycle tests using fathead minnows as the test organism were initiated by Bentley et al. (1978). The first test was terminated after 140 days, while still in the first generation, because of an accidental fish kill. Nevertheless, the results reported up to that point indicated no significant adverse effects of RDX at concentrations as high as 4.9 mg/l on hatchability, 30 day survival and length or on 60-day length and weight. However, a significant ($P \leq 0.05$) reduction in survival after 60 days was observed at a challenge concentration of 4.9 mg/l. In general these results were verified by the second life-cycle test. No significant effect was observed on first generation hatchability, 30-, 60-, and 240-day length or on 240-day fish weights at RDX concentrations as high as 6.3 mg/l. Survival at 30 and 60 days was significantly ($P < 0.05$) reduced with respect to controls at a concentration of 6.3 mg/l. The earlier and greater mortalities observed in the course of this second study may have been due to the higher average RDX concentration (Bentley et al., 1978).

No significant effects were observed on total spawns, number of eggs produced per treatment, number of eggs per spawn or on the hatchability of eggs from exposed parents at RDX concentrations as high as 6.3 mg/l. The number of spawns per female as well as the number of eggs per female at 6.3 mg/l was significantly ($P \leq 0.05$) greater than control. This was attributed to the fact that fewer females at this challenge concentration had survived to the spawning period (Bentley et al., 1978).

No significant effects on survival, length or weight of second generation fry were observed after 30 days exposure to RDX at concentrations as high as 6.3 mg/l.

HMX Toxicology

Freshwater Algae. Bentley et al. (1977) investigated the toxicity of HMX to four species of freshwater algae. Two of these species are eucaryotes: Navicula pelliculosa, a diatom; and Selenastrum capricornutum, a chlorophyte. The other two species, Microcystis aeruginosa and Anabaena flos-aquae, are members of the procaryotic Myxophyceae. Procedures outlined by the EPA (Algal Assay Procedure: Bottle Test, EPA 1971) were used to define the response of five nominal HMX concentrations ranging from 0.32 to 32.0 mg/l plus control. Chlorophyll a and either cell numbers (N. pelliculosa, S. capricornutum and M. aeruginosa) or optical density (A. flos-aquae) measurements were obtained from triplicate exposed cultures.

Bentley et al. (1977) reported 96-hour EC50's greater than 32 mg/l for all four algae species tested. However, their use of the probit transformation to analyze growth response relative to a control is a questionable procedure (Finney 1971). In order to estimate a no-effect concentration, ANOVA was performed on the 96-hour chlorophyll a and cell count (or optical density) data of Bentley et al. (1977). Subsequently, in tests where a significant ($P < 0.05$) response was indicated, a one-sided Dunnett's test as well as Williams' procedure (1972) for comparison of individual treatment means to control were applied to the data. Table 16 summarizes the results of this analysis.

TABLE 16

RESPONSE OF FOUR FRESHWATER ALGAE SPECIES TO HMX

Algae Species Tested	Lowest Significant ($P \leq .05$) Response (Chlorophyll a) Concentration at 96 Hours[c]		Lowest Significant ($P < .05$) Response (Cell Counts[b]) Concentration at 96 Hours[c]	
	Dunnetts[a]	Williams	Dunnetts[a]	Williams
Eucaryotes:				
Navicula pelliculosa	N.S.[d]	N.S.[d]	10.0	10.0
Selenastrum capricornutum	10.0	10.0	10.0	10.0
Procaryotes:				
Microcystis aeruginosa	N.S.[d]	N.S.[d]	N.S.[d]	N.S.[d]
Anabaena flos-aquae	N.S.[d]	N.S.[d]	10.0	10.0

[a]One-tailed test.

[b]Response was determined by cell counts in all species except A. flos-aquae in which response was determined by optical density.

[c]Based on nominal HMX concentrations (mg HMX/l).

[d]Not significant.

HMX concentrations as low as 10 mg/l significantly stimulated the growth of three of the algae species tested; N. pelliculosa (12 percent increase) S. capricornutum (3 percent increase), and A. flos-aquae (7 percent increase) using cell counts (or optical density) as the measure of growth response.

S. capricornutum was the only algae species tested to exhibit a significant increase in chlorophyll a after 96 hours exposure to HMX. The slight effect (10 percent) was significant at a nominal HMX concentration 10 mg/l. The extreme sensitivity of the statistical methods employed in resolving the response level of the algae species tested is due to the low variance of the measurements obtained from the replicated cultures. As with the RDX results, one might question whether or nor the small changes found to be statistically different represent significant biologic differences.

Freshwater Invertebrates. Bentley et al. (1977) conducted static acute bioassays using four species of freshwater invertebrates: the midge Chironomus tentans, Daphnia magna, a Cladoceran, the amphipod Gammarus fasciatus and the isopod Asellus militaris. As a group, invertebrates appear to be less sensitive than either fish or algae to the acute effects of HMX. After 48 hours no adverse effects attributable to HMX toxicity were reported for any species tested, even at the highest challenge concentrations (i.e., 32 mg/l). Thus, only a lower limit based on nominal HMX concentrations can be placed on the 48-hour EC50's for the species tested, i.e., greater than 32 mg/l (see Table 17).

Freshwater Fish. Bentley et al. (1977) conducted static, acute bioassays with HMX using four freshwater fish species: bluegill, Lepomis macrochirus; channel catfish, Ictalurus punctatus; fathead minnow, Pimephales promelas and rainbow trout, Salmo gairdneri. All reported results were based on nominal HMX concentrations.

Table 18 summarizes the acute toxic responses found under static test conditions. The fathead minnow, used to test the acute toxicity of HMX to various life stages under static test conditions was the most sensitive freshwater fish species examined. Susceptibility of this organism was greatest at 7 days post-hatch, having a 96-hour LC50 of 15 mg/l. All other life stages tested exhibited 96-hour LC50's of >32 mg/l, the highest challenge concentration. It should be noted that the higher level nominal concentrations were above the solubility of HMX as reported by Barkley (1977).

The bluegill was used to test the effects of temperature, pH and water hardness on the acute toxicity of HMX. However, none of these water quality parameters, in the ranges tested, significantly affected the sensitivity of the bluegill to HMX.

Mechanisms of Toxicity

The mode of action related to RDX toxicity has been studied to a limited extent in mammals. No information is available for vertebrate or invertebrate aquatic species. That data base available from laboratory

TABLE 17

ACUTE TOXICITY OF HMX TO FRESHWATER INVERTEBRATES
UNDER STATIC TEST CONDITIONS

Organism	Acute Toxic Concentration (mg HMX/l) [a,b]		Reference
	48-Hour EC50	Other	
Freshwater Invertebrates			
Cladoceran *Daphnia magna*	>32	24-hr EC50, >32	Bentley *et al.* (1977)
Amphipod *Gammarus fasciatus*	>32	24-hr EC50, >32	Bentley *et al.* (1977)
Isopod *Asellus militaris*	>32	24-hr EC50, >32	Bentley *et al.* (1977)
Midge *Chironomus tentans*	>32	24-hr EC50, >32	Bentley *et al.* (1977)

[a]EC50 based on immobilization of organism.

[b]Unless otherwise noted, all reported values based on nominal concentrations.

TABLE 18

ACUTE TOXICITY OF HMX TO FRESHWATER FISH
UNDER STATIC TEST CONDITIONS

Organism	Acute Toxic Concentration (mg HMX/l)[a] 96-Hour LC50	Other	Comments[b]	Reference
Freshwater Fish				
Bluegill		24-hr LC50, >32		
Lepomis macrochirus		48-hr LC50, >32		Bentley, et al. (1977)
	>32			
	>32		T=15°C, pH=7.0, TH=35 mg/l $CaCO_3$	
	>32		T=20°C, pH=7.0, TH=35 mg/l $CaCO_3$	
	>32		T=25°C, pH=7.0, TH=35 mg/l $CaCO_3$	
	>32		T=20°C, pH=7.0, TH=35 mg/l $CaCO_3$	
	>32		T=20°C, pH=7.0, TH=100 mg/l $CaCO_3$	
	>32		T=20°C, pH=7.0, TH=250 mg/l $CaCO_3$	
	>32		T=20°C, pH=6.0, TH=35 mg/l $CaCO_3$	
	>32		T=20°C, pH=7.0, TH=35 mg/l $CaCO_3$	
	>32		T=20°C, pH=8.0, TH=35 mg/l $CaCO_3$	
	>32		Initial Age of HMX Test Solution = 0 hr	
	>32		Initial Age of HMX Test Solution = 12 hrs	
	>32		Initial Age of HMX Test Solution = 24 hrs	
	>32		Initial Age of HMX Test Solution = 48 hrs	
	>32		Initial Age of HMX Test Solution = 96 hrs	
Fathead Minnow		24-hr LC50, >32		
Pimephales promelas		48-hr LC50, >32		Bentley, et al. (1977)
	>32			
		24-hr LC50, >32	Eggs	
		48-hr LC50, >32	Eggs	
	>32		Eggs	
		144-hr LC50, >32	Eggs	
		24-hr LC50, >32	1 hr, Post Hatch, Fry	
		48-hr LC50, >32	1 hr, Post Hatch, Fry	
	>32		1 hr, Post Hatch, Fry	
		24-hr LC50, >32	7 Days, Post Hatch, Fry	
		48-hr LC50, 25(7.6-81)	7 Days, Post Hatch, Fry	
	15(8.8-26)		7 Days, Post Hatch, Fry	
		24-hr LC50, >32	30 Days, Post Hatch, Fry	
		48-hr LC50, >32	30 Days, Post Hatch, Fry	
	>32		30 Days, Post Hatch, Fry	
		24-hr LC50, >32	60 Days, Post Hatch, Fry	
		48-hr LC50, >32	60 Days, Post Hatch, Fry	
	>32		60 Days, Post Hatch, Fry	

Continued on following page.

TABLE 18 (continued)

Channel Catfish *Ictalurus punctatus*	>32	24-hr LC50, >32 48-hr LC50, >32		Bentley, *et al.* (1977)
Rainbow Trout *Salmo gairdneri*	>32	24-hr HC50, >32 48-hr HC50, >32		Bentley, *et al.* (1977)

[a]Numbers in parentheses represent 95 percent fiducial/confidence limits

[b]Unless otherwise noted, all reported values based on nominal concentrations

studies in dogs, rats and pigs is useful, however, as certain inferences can be made about toxic mechanisms of this compound.

In the studies reviewed, animals were fed sufficiently large amounts of RDX to produce an acute toxic LD50 response. Following the exposure to RDX, animals most commonly responded with a convulsive reaction. A study carried out by the University of Maryland (1975) indicated that acute high doses of the compound induced signs and symptoms of central nervous system involvement. *In vitro* studies with brain homogenate indicated an inhibition of cholinesterase and monoamine oxidase activity. In addition, the results suggested an inhibition of oxidative phosphorylation as measured by oxygen uptake by the homogenates.

Results of the *in vitro* studies suggested also that enzymatic changes were directly due to RDX itself rather than a metabolite. This would indicate that the convulsive response was due to RDX alone and not to a byproduct or secondary compound formed during the metabolism of RDX by the test animal.

Toxic reactions in the rat and miniature swine were documented in a study carried out by Schneider *et al.* (1976). The convulsive syndrome from high acute dosages were observed in both animals. The pig demonstrated a latent response period more comparable to that observed in humans. The data, however, suggest that the mechanism of toxicity is at least dual and involves the intact RDX molecule as well as a metabolite. The latter is possibly connected with the nitramine grouping in the compound and could act in a manner analogous to nitrosamine.

These researchers point out that acute toxic LD50 response was dependent on the physical form of RDX and on the method used to suspend or dissolve it for administration. Since RDX is only slightly soluble in water, a carrier is frequently used. Reaction time of the compound was shortened in this way. Increase in surface area of a compound by feeding a smaller particle size also resulted in triggering a more immediate acute response. It has been shown that RDX fed to dogs in food pellets over a 13 week period at 10 mg/kg/day had no visible toxic effect. The same dosage introduced as a liquid directly to the stomach produced an acute toxic response manifested by convulsions (Schneider *et al.* 1976).

At present, mammalian work indicates that metabolism of RDX occurs in the liver. Schneider et al. (1976) administered 50 mg ^{14}C-RDX/kg po to rates in a dimethylsulfoxide solution. After four days, 43 percent of the administered radioactivity was recovered as $^{14}C\text{-}CO_2$, 34 percent in the urine, 10 percent in the carcass, 2-3 percent in the feces and 10-11 percent was unaccounted for. Further studies would be required to delineate specific pathways in test animals.

Applicability of the mammalian data to toxicity mechanisms in aquatic organisms can at best only be made by inference. Since there is some commonality of enzyme systems, the assumption can be made that RDX toxicity would effect these systems regardless of species or phylogenetic differences. For either fish or invertebrates chloinesterase or equivalent enzymes related to neural transmission are most probably affected. Exposure to RDX at high levels which causes acute toxicity and death

could result from inhibition of chloinesterase activity. Survival at chronic dosage levels could result from the ability of the enzyme system to function even when partially inhibited.

There is also evidence that oxidative phosphorylation is affected. This would result in an interference of carbohydrate metabolism. There is no indication presently that respiration at the cellular level is involved. It appears that RDX causes little or no cytopathological effects since the mammalian studies did not indicate this effect resulting from either short or long-term exposure.

Presently there is no information related to the metabolic pathway of the nitrogen portion of the RDX molecule. The mammalian data suggests that the nitramine structure may produce a neurotoxic response or may act as an analog to nitrosamine, a recognized carcinogen.

V. Environmental Fate and Effects

The aqueous environmental chemistry of RDX and HMX has been investigated by and/or for the U.S. Army Medical Research and Development Command and the U.S. Navy, Naval Surface Weapons Center. The foci of these investigations are both the environmental fate and the treatability of wastewater containing nitramines. At this time most of the results deal with RDX, especially for studies dealing with the reactions and fate of the nitramines in aquatic ecosystems. Biological degradation, carbon and resin absorption, ion-exchange, as well as chemical oxidation and photolytic decomposition methods of wastewater treatment have also yielded information pertinent to the aquatic chemisty of RDX and HMX.

Removal of RDX from the water column can be effected by precipitation, absorption on sediments, hydrolysis, oxidation photolysis, or microbial action. The only comparisons between the aquatic chemistry of RDX and HMX have been generated in wastewater treatability studies (Jackson et al. 1976; Green 1972; Small and Rosenblatt 1974; Andrews and Osmon 1977; and Jain 1976). These data suggest that RDX and HMX have similar properties in aquatic systems.

Spanggord et al. (1978) studied the relative resistance to degradation of TNT (2,4,6-trinitrotoluene) and RDX in an effort to determine an average ratio which might represent the munitions residues resulting from discharges of composition B and from load, assembly, and pack facilities. RDX appeared to be much more resistant than TNT to removal in studies of wastewaters which passed through a series of ponds at Louisiana Army Ammunition Plant. This was demonstrated by the average TNT/RDX ratios of 1.83 in the pond influent and 0.39 in the effluent.

Hydrolysis. RDX does not hydrolize to an appreciable extent in either freshwater or seawater at neutral or acidic pH values. Hoffsommer and Rosen (1973) found approximately 12 percent of a 56 mg/l RDX solution in seawater degraded in 112 days.

Photolysis. RDX is photolabile, especially by the action of ultraviolet light. This property has been extensively studied as a possible wastewater treatment method (Kubose and Hoffsommer 1977, and Andrews and

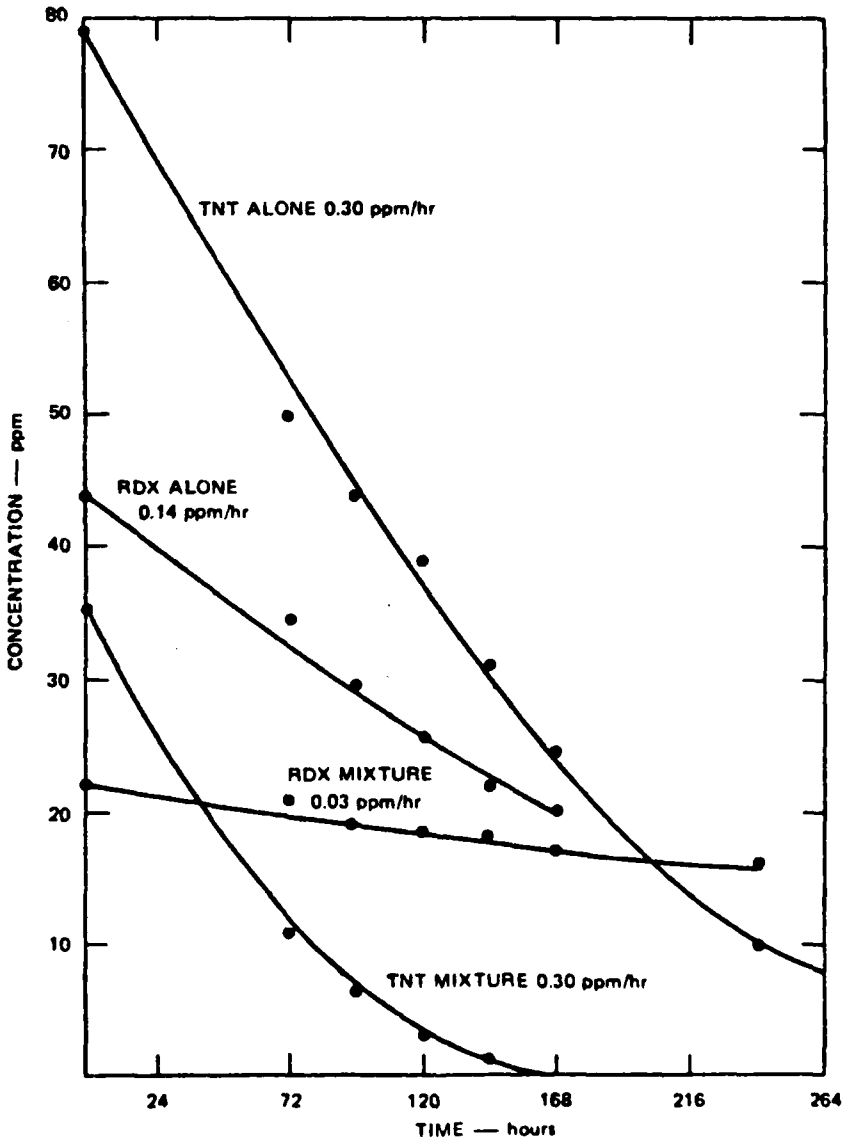
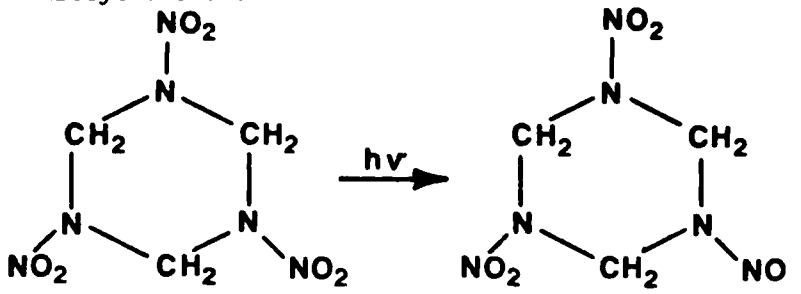


FIGURE 3. DECOMPOSITION OF RDX AND TNT (ALONE AND MIXED) IN SUNLIGHT.
REFERENCE: SPANGGORD ET AL. (1978).

Osmon 1977) and in terms of its environmental fate (Spanggord et al. 1978). Kubose and Hoffsommer (1977) irradiated 20 to 40 mg/l RDX solutions in a shallow (0.5-3 cm) laminar flow system and found that UV light essentially completely degraded the RDX (to less than 0.005 mg/l) in 5 to 10 minutes. Full spectrum UV (>220 nm) and pyrex-glass-filtered UV (>280 nm) light were utilized. The major intermediate breakdown product identified was the mononitroso derivative of RDX, 1-nitroso-3,5-dinitro-1,3,5-triazacyclohexane:



(7)

In addition, other photolabile intermediates formed during the full spectrum UV exposures. None of the products detected persisted after the RDX concentration became undetectable. The rate of photolysis under the filtered UV was much slower than under the full spectrum; however, no data were given on rates under the former treatment. Only small amounts of the intermediates formed. The concentration of the most prevalent (the mononitroso-intermediate) was estimated to be less than 0.1 mg/l or about 0.6 percent of the initial RDX concentration.

Oxygen concentration and pH did not appear to affect photolysis rates; however, photolysis of RDX in distilled water caused the pH to drop to 3-4 in the photolyzed solution. This was attributed to HNO_2 formation. Other products found and their mole ratios are shown below:

Ratio	$\frac{NO_3^-}{RDX}$	$\frac{NO_2^-}{RDX}$	$\frac{CH_2O}{RDX}$	$\frac{NH_3}{RDX}$	$\frac{N_2O}{RDX}$	$\frac{N_2}{RDX}$
>220 nm	trace	2.4	0.8	0.6	0.05-0.1	0.1-0.2
>280 nm	0.7	2.0	0.6	0.7	---	---

The presence of CO_2 in the photolyzed solution was suspected but not confirmed.

Kubose and Hoffsommer (1977) attributed the differences in mole fractions at the different wave lengths to two different mechanisms for the initial photolytic step. The higher energy light (>220 nm) was thought to cleave the N-N bond while the less energetic light was thought to rupture the N-O bond. These data are consistent with the theory that the axial NO_2 groups are the most highly stressed portions of the molecule and would be expected to be most easily broken. Andrews and Osmon (1977) found that UV light completely degraded 5 ppm of HMX within 2 hours.

The presence of TNT inhibits photolysis of RDX. Figure 3 (Spanggord et al. 1978) shows that RDX photolysis is strongly affected by the more

labile TNT molecule. RDX apparently does not influence the rate of TNT photolysis.

Adsorption by Soils. Hale et al. (1978) studied the movement of TNT, Tetryl and RDX in 60" soil columns and found essentially no leaching of RDX residues (<0.05 mg/l) downward through the soil. There was some evidence that the munitions compounds were slowly degraded; however, at the end of six months, ^{14}C analysis suggested that some soluble breakdown products were being formed. The amounts were too low to quantify using hplc.

Microbiological Degradation. Work done by Small and Rosenblatt (1974) and pilot plant studies by Green (1972) suggested that RDX and HMX could be at least partially degraded by aquatic microflora. Green was able to remove 42 percent of the RDX and 53 percent of the HMX from HAAP area B wastes using an activated sludge reactor dominated by Pseudomonas spp. and Alcaligenes spp. In contrast, Soli (1973) concluded that aerobic heterotrophs could not degrade RDX but that photosynthetic anaerobes could. Mixed cultures of Thiorhodaceae and Athiorhodaceae were able to degrade 97 percent of a 20-mg/l RDX solution within 5 days.

More recently, Stilwell et al. (1977) presented RDX and HMX removal data from pilot plant units at HAAP in support of fish bioassay results. Two treatment schemes were utilized: 1) denitrification plus activated sludge and 2) dentrification plus aerobic trickling filter plus activated sludge. Both systems were effective in reducing RDX concentrations. The latter system was more effective in HMX removal.

Field Surveys. A number of field surveys have been performed at HAAP and a number of LAP facilities handling RDX and/or HMX in order to assess the impact of the waste discharges from these facilities on the aquatic environment. However, only the work performed by Sullivan et al. (1977) at HAAP was designed such that a determination of the fate and effects of RDX and HMX in the aquatic environment could be made. In this study, algae and macroinvertebrates were the foci of investigation. Selection of these communities was made because of their short response time to environmental stress, the limited mobility of the attached and burrowing forms as well as the ease with which they colonize an artificial substrate.

The direct impacts of RDX and HMX discharges from HAAP were deemed extremely difficult to assess as a result of the environmental stresses imposed by several extraneous factors. Among the factors stressing the biologic communities in the South Fork of the Holston River and in the Holston River were 1) the highly variable hydrologic flow regime due to intermittent releases from Ft. Patrick Henry Dam on the South Fork of the Holston River, 2) upstream industrial and domestic waste discharges resulting in eutrophic conditions and dissolved oxygen depression, and 3) the highly variable waste discharges released from HAAP. Measured RDX concentrations in the receiving waters ranged from 0.7 mg/l immediately below the outfall to <0.005-0.07 mg/l approximately one mile downstream. No RDX was detected in any of the sediments sampled. HMX was not detected in the receiving waters, precluding an impact evaluation of this compound.

The natural benthic invertebrate community upstream of the HAAP outfalls was characterized as being stress-tolerant. However, impacts on population density and diversity were detected up to ~200 yards downstream of the two upper Area B production outfalls. These effects could not be related to RDX or HMX discharges, however.

Periphyton communities were examined from a selected number of natural substrates as well as artificial substrates placed at several locations in the study area. The periphyton and diatom populations upstream of the influence of the HAAP outfalls were characterized as pollutant tolerant and characteristic of nutrient-enriched waters. The overall effect of the HAAP waste discharges on the sampled periphyton community consisted of a marked increase in heterotroph biomass, reduced autotroph populations and a shift in the diatom species assemblage. The changes in the diatom population structures were correlated with elevated levels of RDX as well as nitrate nitrogen, total Kjeldahl nitrogen and total organic carbon, and were manifested in terms of species replacements since overall diatom species diversity was relatively unaffected. The effects on the periphyton were observed in waters containing RDX concentrations as low as 0.02 mg/l.

In summary, field studies indicate biological effects in waters receiving RDX-and-HMX-contaminated wastewaters. Other factors including the changing chemical environment, the eutrophic conditions and stresses imposed by upstream discharges as well as the possibility of synergistic effects between RDX and/or HMX and other factors indicate a tenuous relationship between RDX and/or HMX and biotic response.

VI. Criteria Development

In developing water quality criteria, all available data were considered. However, the laboratory bioassay results were the only data from which RDX concentrations could be related directly to biologic effects. The approaches outlined below were compared in order to arrive at suitable criteria.

1. The proposed EPA procedure as outlined in the Federal Register (EPA 1978).
2. Traditional approaches described by the American Public Health Association (1975); the National Academy of Sciences (1973); EPA (1976) which develop criteria based on application factors and acute toxicity values (EC or LC50's) for sensitive species.
   a. The lowest LC or EC50 value found multiplied by a conservative application factor. This factor is chosen by experience in order to provide a conversion to chronic effects based on the nature of the toxicant. Where toxicants have a nonpersistent nature or noncumulative effects the 24-hour average concentration should not exceed 0.05 of the LC50 of the most sensitive species tested. Where toxicants are persistent or cumulative, the 24-hour average concentration should not exceed 0.01 of the LC50 of the most sensitive species tested
   b. The lowest LC or EC50 value found multiplied by the lowest experimentally derived application factor.

RDX Criteria

Proposed EPA Procedure. The recently proposed EPA procedure provides a very detailed protocol for evaluating a bioassay data base to determine water quality criteria. Precise procedures have been described for converting data to a common basis and for deriving criteria from the converted data. This procedure may or may not be adopted in its present form. To facilitate the reader's understanding of this procedure, the same paragraph notation will be utilized here as is used in the "Guidelines" section of the EPA procedure. All data are for freshwater.

I. Final Freshwater Fish Acute Value
   A. Data base - (see Tables 13 and 14).
   B. Adjust nominal concentrations by a factor 0.77 to simulate results based on measured concentrations - (see Table 19).
   C. Adjust for reported test results from 24-, 48-, and 72-hour tests by factors of 0.66, 0.81 and 0.92, respectively - (see Table 19).
   D. Adjust values from static tests by a factor of 0.71 to simulate results from flow-through tests (see Table 19).
   E. For each species the geometric mean of the LC50 values (corrected when necessary) is shown in Table 20.
   F. The geometric mean of all species geometric means is 3.9 mg/l.
   G. The final freshwater fish acute value is the lower of the following values:
      1. The geometric mean from item F, above, divided by 3.9, i.e., 1.0 mg/l.
      2. The lowest LC50 value (corrected when necessary) based on measured concentrations from a flow-through test, 2.0 mg/l.

   Therefore, the final freshwater fish acute value for RDX is 1.0 mg/l.

II. Final Freshwater Fish Chronic Value
   A. Chronic values are calculated as the geometric mean of the MATC* limits for life cycle or partial life cycle tests and/or one-half of the geometric mean of the MATC limits derived from embryo-larval tests (see Table 21 for results).
   B. Acceptable chronic values are available for one species as a result of both life cycle and embryo-larval tests.
   C. The freshwater fish chronic value is the lowest of the following values:
      1. The lowest individual chronic value, i.e., 0.95 mg/l.
      2a. For each species the geometric mean of chronic values from A above is determined (see Table 21).
      2b. The geometric mean of the geometric means of all species is determined, i.e., 2.0 mg/l.
      2c. The geometric mean from item 2b above divided by 6.7, i.e., 0.30 mg/l.
      3. This item requires matched pairs of MATC limits and 96-hour LC50 values based on flow-through tests with measured concentration. Since all the LC50 values for flow-through

TABLE 19

DATA BASE FOR FRESHWATER FISH ACUTE VALUE:
RDX - 96-HOUR LC50'S

Species	Test Type of Reported LC50	Reported LC50 (mg/l)	Overall Correction Factor	Corrected LC50 (mg/l)	Reference
Bluegill[a]	Nom., 96-hr, Static	6.0	0.547	3.3	Bentley et al. (1978)
		8.4		4.6	
		5.1		2.8	
		4.1		2.2	
		3.8		2.1	
		5.3		2.9	
		3.9		2.9	
		3.6		2.0	
		3.7		2.0	
		3.9		2.1	
		4.7		2.6	
		4.8		2.6	
		5.1		2.8	
		4.8		2.6	
		4.8		2.6	
	Nom., 96-hr, Flow-thru	7.6	0.77	5.9	
Fathead Minnow[b]	Nom., 96-hr, Static	5.8	0.547	3.2	Bentley et al. (1978)
		>100		---	
		43		24	
		3.8		2.1	
		16		8.7	
		11		6.0	
		5.3		2.9	Liu et al. (1977)
	Nom., 96-hr, Flow-thru	6.6	0.77	5.1	Bentley et al. (1978)
Channel Catfish[c]	Nom., 96-hr, Static	4.1	0.547	2.2	Bentley et al. (1978)
	Nom., 96-hr, Flow-thru	13	0.77	10	
Rainbow Trout[d]	Nom., 96-hr, Static	6.4	0.547	3.5	Bentley et al. (1978)

[a] Lepomis macrochirus

[b] Pimephales promelas

[c] Ictalurus punctatus

[d] Salmo gairdneri

Nom. = Nominal

TABLE 20
COMBINED DATA BASE FOR FRESHWATER FISH ACUTE VALUE:
RDX - 96-HOUR LC50'S

Fish Species	No. of Values	Geometric Mean of All LC50 Values by Species, mg/l
Bluegill[a]	16	2.7
Fathead Minnow[b]	6	5.9
Channel Catfish[c]	2	4.7
Rainbow Trout[d]	1	3.5

[a] Lepomis macrochirus
[b] Pimephales promelas
[c] Ictalurus punctatus
[d] Salmo gairdneri

TABLE 21
DATA BASE FOR FRESHWATER FISH CHRONIC VALUE:
RDX

Life Cycle Tests - Concentrations in mg/l

Fish Species	Response Parameter	MATC Limits	Geometric Mean
Fathead Minnow [a]	30 and 60 Day % Survival	3.0-6.3	4.3

Embryo-Larval Test

Fish Species	Response Parameter	MATC Limits	Geometric Mean	1/2 Geometric Mean
Fathead Minnow [a]	30 Day Length	1.2-3.0	1.9	0.95
Channel Catfish[b]	30 Day % Survival	0.71-1.2[c]	0.92[c]	0.46[c]

Summary of Chronic Value Data

Fish Species	Geometric Mean
Fathead Minnow [a]	2.0
Channel Catfish[b]	0.46[c]

[a] Pimephales promelas

[b] Ictalurus punctatus

[c] Equipment malfunction suspected

tests are based on unmeasured concentrations, no such matched pairs exist.

Therefore, the final freshwater fish chronic value for RDX is 0.30 mg/l.

III. Final Freshwater Invertebrate Acute Value
   A. Data base (see Tables 10 and 11).
   B. Since no definitive 24-, 48-, 72- or 96-hour LC50 values are available for freshwater invertebrates, **no final freshwater invertebrate acute value for RDX can be derived.**

IV. Final Freshwater Invertebrate Chronic Value
   A. Chronic values are calculated as the geometric mean of the MATC limits from life-cycle or partial life-cycle tests (see Table 22).
   B. An acceptable chronic value is available for one species of freshwater invertebrate.
   C. The final freshwater invertebrate chronic value is the lower of the following values:
      1. The lowest chronic value, i.e., 3.2 mg/l.
      2. The geometric mean of the geometric means for all species divided by 5.1, i.e., 3.2/5.1 = 0.63 mg/l.

   Therefore, the final freshwater invertebrate chronic value for RDX is 0.63 mg/l.

V. Final Freshwater Plant Value
   A. Tests were conducted on four species of freshwater algae (see Table 9).
   B. The lowest available plant toxicity value, i.e, the 96-hour no-effect level based on growth inhibition of Selenastrum capricornutum, is indeterminate but is less than 0.32 mg/l. However, the slight 2-3 percent changes observed in organism response to low level RDX challenge concentrations, although declared statistically significant as a result of the low variance of the test measurements and the extreme sensitivity of the statistical procedures employed, are of questionable biological significance. Since the lowest value from item B above is indeterminate, i.e., less than 0.32 mg/l, **no final freshwater plant value can be derived.**

VI. Freshwater Residue Limited Toxicant Concentration (RLTC)
   A. The lowest published lethal dose (LDLo) appearing in the Registry of Toxic Effects of Chemical Substances, 1976, is the oral LDLo for rats, i.e., 200 mg/kg.
   B. Sufficient data are available to establish the RLTC (see Table 23).

*Maximum Acceptable Toxicant Concentration (MATC): the highest concentration of toxicant that has no adverse effect on survival, growth or reproduction of a species based on the results of a life-cycle or partial life-cycle toxicity test. A life-cycle or partial life-cycle test cannot produce a value for the MATC; a test can only produce limits within which the MATC must fall.

TABLE 22
DATA BASE FOR FRESHWATER INVERTEBRATE CHRONIC VALUE:
RDX

Life-Cycle Tests - Concentrations in mg/l

Invertebrate Species	Response Parameter	MATC Limits	Geometric Mean
Daphnia magna	1st generation, 14-Days; number of young per female	2.2-4.8	3.2

TABLE 23

BIOCONCENTRATION (BCF) OF RDX IN TISSUES OF FRESHWATER FISH

Fish Species	BCF[a] (For Exposure Duration Greater Than 27 Days)			Species Average	Reference
	Tissue	Reported Range	Average		
Fathead Minnow[b]	Edible	4.0-5.9	4.95	4.95	Bentley et al. (1978)
Channel Catfish[c]	Edible	2.9-4.0	3.45	3.45	Bentley et al. (1978)
Bluegill[d]	Edible	3.5-4.7	4.1	4.1	Bentley et al. (1978)

[a]BCF = Bioconcentration Factor

[b]*Pimephales promelas*

[c]*Ictalurus punctatus*

[d]*Lepomis macrochirus*

C. The arithmetic average bioconcentration factor BCF of all freshwater fish species arithmetic averages is 4.2.
D. Not applicable.
E. The freshwater RLTC is the lowest permissible tissue concentration from item A above divided by the measured bioconcentration factor from item C above, i.e., 200/4.2 = 47 mg/l.

VII. Other Data
Other data will be considered in other criteria setting procedures which follow.

VIII. Final Freshwater Values
A. The "final freshwater acute value" is the final freshwater fish acute value since no acceptable freshwater invertebrate acute value is available.
B. The "final freshwater chronic value" is the lowest of the following values:
1. The final freshwater fish chronic value, i.e., 0.30 mg/l.
2. The final freshwater invertebrate chronic value, i.e., 0.63 mg/l.
3. The final freshwater plant value, which is indeterminant but is less than 0.32 mg/l.
4. The freshwater RLTC, i.e., 47 mg/l.

Therefore, the "final freshwater chronic value" for RDX is 0.30 mg/l.

IX. Freshwater Criterion
A. Sufficient data are available to establish a criterion under this guideline.
B. The maximum freshwater concentration should never exceed the final freshwater acute value, i.e., 1.0 mg/l.
The 24-hour average concentration should never exceed the lower of the following values:
1. The "final freshwater acute value" from item VIIIA above, times 0.44, i.e., (1.0)(0.44) = 0.44 mg/l.
2. The "final freshwater chronic value" from item VIIIA above, i.e., 0.30 mg/l.

Therefore, the 24-hour average RDX concentration should not exceed 0.30 mg/l.

Traditional Approach. Traditionally, environmentally safe concentrations have been derived when only acute toxicity data are available by use of an application factor. This factor is applied to the lowest acute (96-hour LC50) toxicity value. A general factor may be utilized or a factor may be experimentally derived.

General Application Factor - Chronic toxicity testing indicates that RDX is not cumulatively toxic. Therefore, by this procedure an application factor of 0.05 would be used. Applying this factor to the lowest acute toxicity result, 3.6 mg/l for bluegill, gives a safe concentration of 0.18 mg/l.

Experimentally Derived Application Factor - A third procedure for deriving environmentally safe concentrations is similar to the previously discussed procedure except that the application factor is experimentally derived. Where both acute and chronic data are available, the application factor is the ratio of the lower MATC limit to the lowest EC50 or LC50 for that species. If data for more than one species exist, the minimum application factor is utilized. This factor, multiplied by the lowest acute EC50 or LC50 value for any species of a comparable taxonomic grouping, i.e. fish/fish, invertebrate/invertebrate, etc., determines an environmentally safe concentration.

The experimentally derived application factor is shown in Table 24. Only one application factor based on conclusive acute toxicity data and partial or complete life-cycle toxicity tests can be derived, i.e., 0.32. Applying this application factor to the lowest acute toxicity result, 3.6 mg/l for bluegill, a value of 1.2 mg/l is obtained as a maximum safe concentration for RDX.

Recommended Criteria. The three procedures for determining an environmentally safe concentration for RDX give the following results.

Procedure	24 hour Average Concentration, mg/l
Proposed EPA	0.30
General Application Factor	0.18
Experimentally Derived Application Factor	1.2

In the toxicity testing done by Bentley et al (1978) the most sensitive result was observed in a 30-day egg-fry study in which length of fathead minnows was reduced at a concentration of 3.0 mg/l (no effect at 1.2 mg/l). However, in subsequent longer term chronic testing the lowest effect level observed was at 6.3 mg/l (no effect at 3.0 mg/l). Since only one species was tested extensively, some margin of safety is needed to protect potentially more sensitive species. The criteria resulting from use of the proposed EPA procedure would offer a safety factor of 4 or 10 depending on what is believed to be the most valid no-effect concentration. This appears reasonable and a 24-hour average concentration of 0.30 mg/l RDX should adequately protect aquatic life.

HMX Criteria

Proposed EPA Procedure. The recent EPA procedure provides a very detailed protocol for evaluating a bioassay data base to determine water quality criteria. Precise procedures have been described for converting data to a common basis and for deriving criteria from converted data. This procedure has been proposed by the EPA and may or may not be adopted. To facilitate the reader's understanding of this procedure, the same paragraph notation will be utilized here as is used in the "Guidelines" section of the EPA procedure.

I. Final Freshwater Fish Acute Value
   A. Data base (see Table 18).

TABLE 24

EXPERIMENTALLY DERIVED APPLICATION FACTORS - RDX

Species	Lowest Reported Acute Value		Chronic Value		Application Factor
	Test Type of LC50	LC50 (mg/l)	Response Parameter	MATC Limits (mg/l)	
Fathead Minnow[a]	Nominal, 96-hr., Static	3.8	Egg-Fry 30 Day Test; 30 Day Length	1.2-3.0	0.32

[a] Pimephales promelas

B. Adjust nominal concentrations by a factor 0.77 to simulate results based on measured concentrations (see Table 25).
C. Adjust for reported test results from 24-, 48-, and 72-hour tests by factors of 0.66, 0.81, and 0.92, respectively (see Table 25).
D. Adjust values from static tests by a factor of 0.71 to simulate results from flow-through tests (see Table 25).
E. For each species the geometric mean of the LC50 values (corrected when necessary) is shown in Table 26.
F. The geometric mean of all species geometric means is 8.2 mg/l.
G. The final freshwater fish acute value is the lower of the following values:
    1. The geometric mean from item F, above, divided by 3.9, i.e., 2.1 mg/l.
    2. The lowest LC50 value (corrected when necessary) based on measured concentrations from a flow-through test, 8.2 mg/l.

Therefore, the final freshwater fish acute value for HMX is 2.1 mg/l.

II. Final Freshwater Fish Chronic Value
A. Chronic values are calculated as the geometric mean of the MATC limits for life-cycle or partial life cycle tests, and/or one-half of the geometric mean of the MATC limits derived from embryo-larvae tests.
B. No acceptable chronic values exist for freshwater fish, therefore, **no final freshwater fish chronic value can be derived.**

III. Final Freshwater Invertebrate Acute Value
A. Data base (see Table 17).

B. Since no acceptable 24-, 48-, 72-, or 96-hour LC50 values are available for freshwater invertebrates, **no final freshwater invertebrate acute values can be derived.**

IV. Final Freshwater Invertebrate Chronic Value
A. Chronic values are calculated as the geometric mean of the MATC from life-cycle or partial life-cycle tests.
B. No acceptable chronic values exist for freshwater invertebrates, therefore, **no final freshwater invertebrate chronic value can be derived.**

V. Final Freshwater Plant Value
A. (Tests were conducted on four species of freshwater algae (see Table 16).
B. The lowest available plant toxicity, value, i.e., the 96-hour no-effect level based on growth stimulation of S. capricornutum and N. pelliculosa and the growth inhibition of A. flos-aquae is 3.2 mg/l.

Therefore, the final freshwater plant value for HMX is 3.2 mg/l.

TABLE 25

DATA BASE FOR FRESHWATER FISH ACUTE VALUE
HMX - 96-HOUR LC50's

Species	Test Type of Reported LC50	Reported LC50 (mg/l)	Overall Correction Factor	Corrected LC50 (mg/l)	Reference
Bluegill[a]	Nom., 96-hr, Static	>32	0.547	---	Bentley et al. (1977)
		>32		---	
		>32		---	
		>32		---	
		>32		---	
		>32		---	
		>32		---	
		>32		---	
		>32		---	
		>32		---	
		>32		---	
		>32		---	
		>32		---	
		>32		---	
		>32		---	
Fathead Minnow[b]	Nom., 96-hr, Static	>32	0.547	---	Bentley et al. (1977)
		>32		---	
		>32		---	
		15		8.2	
		>32		---	
		>32		---	
Channel Catfish[c]	Nom., 96-hr, Static	>32	0.547	---	Bentley et al. (1977)
Rainbow Trout[d]	Nom., 96-hr, Static	>32	0.547	---	Bentley et al. (1977)

[a] Lepomis macrochirus

[b] Pimephales promelas

[c] Ictalurus punctatus

[d] Salmo gairdneri

Nom. = Nominal

TABLE 26

COMBINED DATA BASE FOR FRESHWATER FISH ACUTE VALUE:
HMX - 96-HOUR LC50's

Fish Species	Number of Values	Geometric Mean of all LC50 Values by Species mg/l
Fathead Minnow [a]	1	8.2

[a] Pimephales promelas

VI. Freshwater Residue Limited Toxicant Concentration (RLTC)
Insufficient data base.

VII. Other Data
Other data will be considered in other criteria setting procedures which follow.

VIII. Final Freshwater Values
A. The "final freshwater acute value" is the final freshwater fish acute value since no acceptable freshwater invertebrate acute value is available, i.e., 2.1 mg/l.
B. The "final freshwater chronic value" is the lowest of the following values:
1. The final freshwater chronic value.
2. The final freshwater invertebrate value.
3. The final freshwater plant value, i.e., 3.2 mg/l.
4. The freshwater RLTC.

Therefore, the "final freshwater chronic value" for HMX is 3.2 mg/l.

IX. Freshwater Criterion
A. Sufficient data are available to establish a criterion under this guideline.
B. The maximum freshwater concentration should never exceed the final freshwater acute value, i.e., 2.1 mg/l.
The 24-hour average concentration should never exceed the lower of the following values:
1. The "final freshwater acute value" from item VIIIA above times 0.44, i.e., (2.1)(0.44) = 0.92 mg/l.
2. The "final freshwater chronic value" from item VIIIB above, i.e., 3.2 mg/l.

Therefore, the 24-hour average HMX concentration should not exceed 0.92 mg/l.

Traditional Approach. Traditionally, environmentally safe concentrations have been derived when only acute toxicity data are available by use of an application factor. This factor is applied to the lowest acute (96-hour LC50) toxicity value. A general factor may be utilized or a factor may be experimentally derived.

General Application Factor - Little data is available regarding the persistence or cumulative nature of HMX. In the absence of such information, the most conservative application factor of 0.01 would be used. Applying this factor to the lowest 96-hour LC50 for fathead minnow, 15 mg/l, gives a safe concentation of 0.15 mg/l HMX (24-hour average).

Experimentally Derived Application Factor - Since no chronic test data is available, an application factor cannot be experimentally derived.

Recommended Criteria. Two of three procedures for determining environmentally safe concentrations of HMX yielded specific results.

Procedure	24-hour Average Concentration mg/l
Proposed EPA	0.92
General Application Factor	0.15
Experimentally Derived Application Factor	Insufficient data

In acute toxicity testing with fish all tests except one showed 96-hour LC50 values of greater than 32 mg/l. Seven day old fathead minnows showed a 96-hour LC50 of 15 mg/l. This concentration is above the solubility of HMX at the test temperature. The algal data showed slight stimulation (3 and 12 percent) to two species and slight inhibition (-7 percent) to one species at 10 mg/l. For a fourth species no effect was observed at 32 mg/l. The biological significance of such changes in algal populations is questionable. For invertebrates, four species all showed 48-hour EC50 values of greater than 32 mg/l. Given that this constitutes the entire data base for HMX toxicity, it is concluded that insufficient data exists to establish criteria to protect aquatic life.

VII. References

American Public Health Association; American Water Works Association; and Water Pollution Control Federation. 1975. Standard methods for the examination of water and wastewater. 14th edition. APHA. Washington, D.C.

Andrews, C.C.; and J.L. Osmon. 1977. The effects of UV light on TNT and other explosives in aqueous solution. Weapons Quality Engineering Center. Naval Weapons Support Center, Crane, Indiana.

Bachmann, W.E. and J.C. Sheehan. 1949. A new method of preparing the high explosive RDX. J. Amer. Chem. Soc. 71: 1842.

Barkley, Jr., J.J. 1977. Determination of the solubility of 1,3,5,7-tetranitrooctahydro-1,3,5,7-tetrazocine (HMX) in water and acetone/water. Memorandum To: J.G. Pearson and M.C. Warner, U.S. Army Medical Research and Development Command, Ft. Detrick, Frederick, MD.

Bentley, R.E.; J.W. Dean; S.J. Ellis; T.A. Hollister; G.A. LeBlanc; S. Sauter; and B.H. Sleight, III. 1977. Laboratory evaluation of the toxicity of cyclotrimethylenetrinitramine (RDX) to aquatic organisms. Final Report. U.S. Army Medical Research and Development Command. Contract No. DAMD-17-74-C-4101. EG&G. Bionomics, Wareham, Massachusetts.

Bentley, R.E.; G.A. LeBlanc; T.A. Hollister; and B.H. Sleight, III. 1977A. Acute toxicity of 1,3,5,7-tetranitrooctahydro-1,3,5,7-tetrazocine (HMX) to aquatic organisms. Final Report. U.S. Army Medical Research and Development Command. Contract No. DAMD-17-74-C-4101. EG&G Bionomics, Wareham, Massachusetts.

Castorina, T.C.; J.W. McCahill; and A.C. Forsyth. 1977. Compatibility of flocculating agents with RDX/TNT/Comp. B. Picatinny Arsenal, Dover, New Jersey.

Cook, M.A. 1958. The science of high explosives. Reinhold Publishing Corp., New York. 440 pp.

Dunnett, C.W. 1955. A multiple comparisons procedure for comparing several treatments with a control. J. Amer. Stat. Assoc. 50:1096-1121.

Environmental Protection Agency. 1971. Algal assay procedure bottle test. National Eutrophication Research Program. Pacific Northwest Laboratory, Corvallis, Oregon.

Environmental Protection Agency. 1976. Quality criteria for water. Environmental Protection Agency, Washington, D.C. EPA440/9/76/023.

Environmental Protection Agency. 1978. Guidelines for deriving water quality criteria for the protection of aquatic life. Ed. T.C. Jorling. Federal Register 43(97). 21506-21518.

Finney, D.J. 1971. Probit analysis. 3rd ed. Cambridge University Press. Cambridge. 333 pp.

Glennon, J.P.; J.G. Pearson; J.C. Dacre; M.C. Warner; and D.H. Rosenblatt. 1977. Munitions environmental quality standards research status report. U.S. Army Medical Bioengineering Research and Development Laboratory Technical Report 7709. Fort Detrick, Frederick, Maryland.

Green, J.M. 1972. Biodegradation of selected nitramines and related pollutants. M.S. Thesis. East Tennessee State University.

Hale, V.Q.; T.B. Stanford; and L.G. Taft. 1978. Fate of munition compounds in soil. U.S. Army Medical Bioengineering Research and Development Command Contract No. DAMD-17-76-6065. Battelle, Columbus, Ohio.

Hawley, G.D. 1977. The condensed chemical dictionary. 9th ed. Van Nostrand-Reinhold.

Hoffsommer, J.C.; and J.M. Rosen. 1973. Hydrolysis of explosives in sea-water. Bull. Environ. Contam. Toxicol. 10. 78-79.

Hoffsommer, J.C. and D.A. Kubose. 1977. Chemical degradation of RDX on strongly basic ion exchange resins. Report No. NSWC/WOL/TR-77-30. Naval Surface Weapons Center. Silver Spring, Maryland.

Hoffsommer, J.C.; D.A. Kubose; and D.J. Glover. 1977. Kinetic isotope effects and intermediate formation for the aqueous alkaline homogeneous hydrolysis of 1,3,5-triaza-1,3,5-trinitrocyclohexane (RDX). In: Journal of Physical Chemistry, 81, 380.

Jackson, R.A.; J.M. Green; and R.L. Hash. 1976. Nitramine removal study. Holston Defense Corporation Contract No. DAAA 09-73-C-0079. Kingsport, Tennessee.

Jain, K.K. 1976. Laboratory study on feasibility of munitions waste-water treatment by adsorption-oxidation. U.S. Army Mobility Equipment Research and Development Center. Fort Belvoir, Virginia.

Kitchens, J.F.; W.E. Harward, III; D.M. Lautar; R.S. Wentsel; and R.S. Valentine. 1978. Final Report. U.S. Army Medical Research and Development Command Contract No. DAMD-17-77-Q-7057. Atlantic Research Corporation, Alexandria, Virginia.

Kubose, D.A.; and J.C. Hoffsommer. 1977. Photolysis of RDX in aqueous solution. Initial studies. Naval Surface Weapons Center. White Oak Laboratory, Silver Spring, Maryland.

Leach, J.T.; and R.L. Hash. 1972. Evaluation of analytical methods for determining RDX, HMX, and TNT in industrial wastewaters. Holston Defense Corp. Report ASQ-3.

Liu, D.H.W.; G.W. Newell; D.J. Spanggord; and H.C. Bailey. 1977. Toxicity of TNT wastewaters to aquatic organisms. Annual Report, January 1976-April 1977. U.S. Army Medical Research and Development Command Contract No. DA,D-17-75-C-5056. Stanford Research Institute, Menlo Park, California.

Mark, H.F.; J.J. McKetta, Jr.; and D.E. Othmer, eds. 1965. In: Kirk-Othmer encyclopedia of chemical technology, 2nd ed. Vol. 8. Interscience, Inc.

Merck and Co., Inc. 1976. The Merck index. An encyclopedia of chemicals and drugs. 9th ed. Merck & Co., Inc., Rahway, New Jersey.

National Academy of Sciences. 1973. Water quality criteria: 1972. Environmental Protection Agency publication, EPA-R3-73-033.

Newell, G.W.; J.V. Dilley; and R.J. Spanggord. 1976. Mammalian toxicological evaluations of TNT wastewater (pink water). Final Report. U.S. Army Medical Research and Development Command Contract No. DAMD-17-74-C-4115. Stanford Research Institute, Menlo Park, California.

Patterson, J.W.; N.I. Shapira; J. Brown; W. Duckert; and J. Polson. 1976. State-of-the-art: military explosives and propellants production industry. Vol. 2. Wastewater characterization. U.S. Environmental Protection Agency Report 600/2-76-213c.

Prestia, J.V. 1975. Polarography of ordinance compounds. Naval Material Command. Office of Support Technology, Washington, D.C.

Schneider, N.R.; S.L. Bradley; and M.E. Anderson. 1976. Toxicology of cyclotrimethylenetrinitramine (RDX): distribution and metabolism in the rat and miniature swine. Armed Forces Radiobiology Research Institute. U.S. Naval Medical Research and Development Command, Bethesda, Maryland.

Small, M.J. and D.H. Rosenblatt. 1974. Munitions production products of potential concern as waterborne pollutants - phase II. Technical Report 7404. U.S. Army Medical Bioengineering Research and Development Laboratory. Aberdeen Proving Ground, Maryland.

Soli, G. 1973. Microbial degradation of cylclonite (RDX). Naval Weapons Center, NWCTP. 5525.

Spanggord, D.J.; B.W. Gibson; R.G. Keek; and G.W. Newell. 1978. Mammalian toxicological evaluation of TNT wastewaters. Vol. 1. Chemistry Studies. U.S. Army Medical Research and Development Command Contract No. DAMD-17-76-C-6050. Stanford Research Institute, Menlo Park, California.

Stals, J. 1969A. The chemistry of aliphatic unconjugated nitramines. II. intramolecular properties and crystal packing. Australian J. Chemistry, 22. 2505-2514.

Stals, J. 1969B. The chemistry of aliphatic unconjugated nitramines III. thermal, electrophilic, and nucleophilic reactions. Australian J. Chemistry, 22. 2515-2526.

Stilwell, J.M.; M.A. Eischen; W.L. Margard; M.C. Matthews; and T.B. Stanford. 1977. Toxicological investigations of pilot treatment plant wastewaters at Holston Army Ammunition Plant. Final Report. U.S. Army Medical Research and Development Command Contract No. DAMD-17-74-C-4123. Battelle. Columbus, Ohio.

Sullivan, J.H., Jr.; H.D. Putnam; M.A. Keirn; D.R. Swift; and B.C. Pruitt, Jr. 1977. Aquatic field surveys at Holston Army Ammunition Plant, Kingsport, Tennessee. U.S. Army Medical Research and Development Command Contract No. DAMD-17-75-C-5049. Washington, D.C.

Szachta, J.M. 1978. Analysis of carbon versus resin. Chemical Systems Laboratory, Aberdeen Proving Ground, Maryland.

University of Maryland. 1975. Acute and chronic biochemical and behavorial effects of RDX. University of Maryland School of Pharmacy (author not stated) Contract No. AD-A 024,415. Office of Naval Research.

Urbanski, T. 1964. Chemistry and technology of explosives. I. The MacMillan Co. New York. 633 pp.

Urbanski, T. 1967. Chemistry and technology of explosives. III. Pergamon Press. Warsaw. 717 pp.

Vlahakis, J.G. 1974. A laboratory study of RDX absorption by carbon. Army Equipment Research and Development Command. Naval Facilities Engineering Command, Alexandria, Virginia.

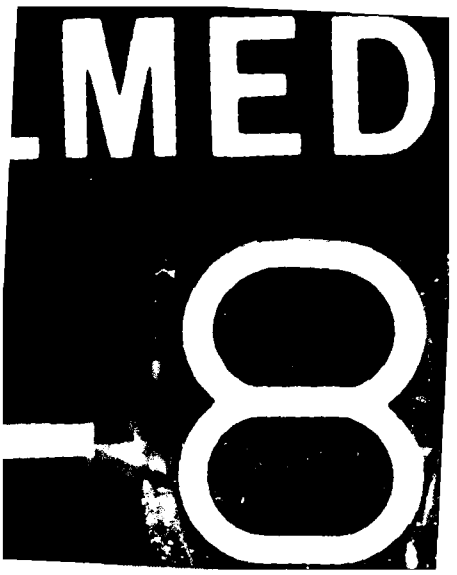
Ward, F.P. and E.S. Bender. 1975. Results of preliminary survey of Milan Army Ammunition Plant (MAAP), Tennessee, 24-26 November 1975. Edgewood Arsenal. Aberdeen Proving Ground, Maryland.

Whitnack, G.C. 1976. Analysis of RDX and HMX in admixture by single sweep polarography. Naval Weapons Center, Gina Lake, California.

Williams, D.A. 1971. A test for differences between treatment means when several dose levels are compared with a zero dose control. Biometrics 27. 103-117.

DISTRIBUTION LIST

25 copies	Commander US Army Medical Bioengineering Research and Development Laboratory ATTN: SGRD-UBG Fort Detrick, Frederick, MD 21701
4 copies	HQDA (SGRD-SI) Fort Detrick Frederick, MD 21701
12 copies	Defense Technical Information Center (DTIC) ATTN: DTIC-DDA Cameron Station Alexandria, Virginia 22314
1 copy	Dean *School of Medicine* Uniformed Services University of the Health Sciences 4301 Jones Bridge Road Bethesda, Maryland 20014
1 copy	Superintendent Academy of Health Sciences, US Army ATTN: AHS-COM Fort Sam Houston, Texas 78234
1 copy	Librarian US Army Medical Bioengineering Research and Development Laboratory ATTN: SGRD-UBG Fort Detrick, Frederick, MD 21701


MED
8